

# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/IB05/002476

International filing date: 07 February 2005 (07.02.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US  
Number: 60/599,014  
Filing date: 06 August 2004 (06.08.2004)

Date of receipt at the International Bureau: 22 July 2005 (22.07.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland  
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1344132

# THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

*July 12, 2005*

**THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.**

**APPLICATION NUMBER: 60/599,014**

**FILING DATE: August 06, 2004**

**RELATED PCT APPLICATION NUMBER: PCT/US05/03826**



Certified by

Under Secretary of Commerce  
for Intellectual Property  
and Director of the United States  
Patent and Trademark Office



16623 U.S. PTO

Approved for use through 10/31/2002. OMB 0651-0032  
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

**PROVISIONAL APPLICATION FOR PATENT COVER SHEET**

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Express Mail Label No.

**INVENTOR(S)**

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Michael	Tesar	Weilheim, Germany

☐ Additional inventors are being named on the \_\_\_\_\_ separately numbered sheets attached hereto**TITLE OF THE INVENTION (500 characters max)**

Anti-CD38 Human Antibodies and Uses Therefor

**CORRESPONDENCE ADDRESS**

Direct all correspondence to:

☒ Customer Number

26633

Place Customer Number  
Bar Code Label here

OR

Type Customer Number here

☐ Firm or  
Individual Name

Address

Address

City

State

ZIP

Country

Telephone

Fax

**ENCLOSED APPLICATION PARTS (check all that apply)**☒ Specification Number of Pages

65

☐ CD(s), Number☒ Drawing(s) Number of Sheets

23

☐ Other (specify)☒ Application Data Sheet. See 37 CFR 1.76**METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT**☒ Applicant claims small entity status. See 37 CFR 1.27.☐ A check or money order is enclosed to cover the filing feesFILING FEE  
AMOUNT (\$)☒ The Commissioner is hereby authorized to charge filing  
fees or credit any overpayment to Deposit Account Number:

08-1641

\$80

☐ Payment by credit card. Form PTO-2038 is attached.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.☐ Yes, the name of the U.S. Government agency and the Government contract number are: \_\_\_\_\_

Respectfully submitted,

SIGNATURE

Date

8/6/04

REGISTRATION NO.  
(if appropriate)

40,244

TYPED or PRINTED NAME

Paul M. Booth

Docket Number:

37629-0085C

TELEPHONE

202-912-2000

2264 U.S. PTO  
60/599014

<h2 style="margin: 0;">FEE TRANSMITTAL</h2> <h3 style="margin: 0;">for FY 2003</h3> <p style="margin: 0; font-size: small;">Effective 01/01/2003. Patent fees are subject to annual revision.</p>		<b>Complete if Known</b>	
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27		Application Number	Unassigned
<b>TOTAL AMOUNT OF PAYMENT</b> (\$) 80.00		Filing Date	Concurrently Herewith
		First Named Inventor	Michael TESAR
		Examiner Name	Unassigned
		Art Unit	Unassigned
		Attorney Docket No.	37629-0085C

<p style="text-align: center; font-weight: bold; font-size: small;">METHOD OF PAYMENT (check one)</p> <p> <input type="checkbox"/> Check                    <input type="checkbox"/> Credit card                    <input type="checkbox"/> Money Order                    <input type="checkbox"/> Other                    <input type="checkbox"/> None             </p> <p><input checked="" type="checkbox"/> Deposit Account:</p> <p>Deposit Account Number: <span style="border: 1px solid black; padding: 2px;">08-1641 (Docket No. 37629-0085C)</span></p> <p>Deposit Account Name: <span style="border: 1px solid black; padding: 2px;">Heller Ehrman White &amp; McAuliffe LLP</span></p> <p>The Commissioner is authorized to: (check all that apply)</p> <p> <input checked="" type="checkbox"/> Charge fee(s) indicated below                    <input type="checkbox"/> Credit any overpayments             </p> <p> <input type="checkbox"/> Charge any additional fee(s) during the pendency of this application             </p> <p> <input type="checkbox"/> Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.             </p> <hr/> <p style="text-align: center; font-weight: bold; font-size: small;">FEE CALCULATION</p> <div style="border: 1px solid black; padding: 5px;"> <p><b>1. BASIC FILING FEE</b></p> <table style="width: 100%; font-size: x-small;"> <thead> <tr> <th>Large Fee Code</th> <th>Entity Fee (\$)</th> <th>Small Fee Code</th> <th>Entity Fee (\$)</th> <th>Fee Description</th> <th>Fee Paid</th> </tr> </thead> <tbody> <tr> <td>1001</td> <td>770</td> <td>2001</td> <td>385</td> <td>Utility filing fee</td> <td></td> </tr> <tr> <td>1002</td> <td>340</td> <td>2002</td> <td>170</td> <td>Design filing fee</td> <td></td> </tr> <tr> <td>1003</td> <td>530</td> <td>2003</td> <td>265</td> <td>Plant filing fee</td> <td></td> </tr> <tr> <td>1004</td> <td>770</td> <td>2004</td> <td>385</td> <td>Reissue filing fee</td> <td></td> </tr> <tr> <td>1005</td> <td>160</td> <td>2005</td> <td>80</td> <td>Provisional filing fee</td> <td>80</td> </tr> <tr> <td colspan="5" style="text-align: right;"><b>SUBTOTAL (1)</b></td> <td style="border: 1px solid black; text-align: right;">(\$) 80</td> </tr> </tbody> </table> <p><b>2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE</b></p> <table style="width: 100%; font-size: x-small;"> <thead> <tr> <th>Total Claims</th> <th>Extra Claims</th> <th>Fee from below</th> <th>Fee Paid</th> </tr> </thead> <tbody> <tr> <td>Independent Claims</td> <td>-20** = 0</td> <td>x</td> <td>0</td> </tr> <tr> <td>Multiple Dependent</td> <td>-3** = 0</td> <td>x</td> <td>0</td> </tr> </tbody> </table> <div style="border: 1px solid black; padding: 5px;"> <table style="width: 100%; font-size: x-small;"> <thead> <tr> <th>Large Fee Code</th> <th>Entity Fee (\$)</th> <th>Small Fee Code</th> <th>Entity Fee (\$)</th> <th>Fee Description</th> </tr> </thead> <tbody> <tr> <td>1202</td> <td>18</td> <td>2202</td> <td>9</td> <td>Claims in excess of 20</td> </tr> <tr> <td>1201</td> <td>86</td> <td>2201</td> <td>43</td> <td>Independent claims in excess of 3</td> </tr> <tr> <td>1203</td> <td>290</td> <td>2203</td> <td>145</td> <td>Multiple dependent claim, if not paid</td> </tr> <tr> <td>1204</td> <td>86</td> <td>2204</td> <td>43</td> <td>**Reissue independent claims over original patent</td> </tr> <tr> <td>1205</td> <td>18</td> <td>2205</td> <td>9</td> <td>**Reissue claims in excess of 20 and over original patent</td> </tr> <tr> <td colspan="5" style="text-align: right;"><b>SUBTOTAL (2)</b></td> </tr> </tbody> </table> </div> <p style="font-size: x-small;">**or number previously paid, if greater; For Reissues, see above</p> </div>	Large Fee Code	Entity Fee (\$)	Small Fee Code	Entity Fee (\$)	Fee Description	Fee Paid	1001	770	2001	385	Utility filing fee		1002	340	2002	170	Design filing fee		1003	530	2003	265	Plant filing fee		1004	770	2004	385	Reissue filing fee		1005	160	2005	80	Provisional filing fee	80	<b>SUBTOTAL (1)</b>					(\$) 80	Total Claims	Extra Claims	Fee from below	Fee Paid	Independent Claims	-20** = 0	x	0	Multiple Dependent	-3** = 0	x	0	Large Fee Code	Entity Fee (\$)	Small Fee Code	Entity Fee (\$)	Fee Description	1202	18	2202	9	Claims in excess of 20	1201	86	2201	43	Independent claims in excess of 3	1203	290	2203	145	Multiple dependent claim, if not paid	1204	86	2204	43	**Reissue independent claims over original patent	1205	18	2205	9	**Reissue claims in excess of 20 and over original patent	<b>SUBTOTAL (2)</b>					<p style="text-align: center; font-weight: bold; font-size: small;">FEE CALCULATION (continued)</p> <p><b>3. ADDITIONAL FEES</b></p> <table style="width: 100%; font-size: x-small;"> <thead> <tr> <th>Large Fee Code</th> <th>Entity Fee (\$)</th> <th>Small Fee Code</th> <th>Entity Fee (\$)</th> <th>Fee Description</th> <th>Fee Paid</th> </tr> </thead> <tbody> <tr> <td>1051</td> <td>130</td> <td>2051</td> <td>65</td> <td>Surcharge - late filing fee or oath</td> <td></td> </tr> <tr> <td>1052</td> <td>50</td> <td>2052</td> <td>25</td> <td>Surcharge - late provisional filing fee or cover sheet</td> <td></td> </tr> <tr> <td>1053</td> <td>130</td> <td>1053</td> <td>130</td> <td>Non-English specification</td> <td></td> </tr> <tr> <td>1812</td> <td>2,520</td> <td>1812</td> <td>2,520</td> <td>For filing a request for <i>ex parte</i> reexamination</td> <td></td> </tr> <tr> <td>1804</td> <td>920*</td> <td>1804</td> <td>920*</td> <td>Requesting publication of SIR prior to Examiner action</td> <td></td> </tr> <tr> <td>1805</td> <td>1,840*</td> <td>1805</td> <td>1,840*</td> <td>Requesting publication of SIR after Examiner action</td> <td></td> </tr> <tr> <td>1251</td> <td>110</td> <td>2251</td> <td>55</td> <td>Extension for reply within first month</td> <td></td> </tr> <tr> <td>1252</td> <td>420</td> <td>2252</td> <td>210</td> <td>Extension for reply within second month</td> <td></td> </tr> <tr> <td>1253</td> <td>950</td> <td>2253</td> <td>475</td> <td>Extension for reply within third month</td> <td></td> </tr> <tr> <td>1254</td> <td>1,480</td> <td>2254</td> <td>740</td> <td>Extension for reply within fourth month</td> <td></td> </tr> <tr> <td>1255</td> <td>2,010</td> <td>2255</td> <td>1,005</td> <td>Extension for reply within fifth month</td> <td></td> </tr> <tr> <td>1401</td> <td>330</td> <td>2401</td> <td>165</td> <td>Notice of Appeal</td> <td></td> </tr> <tr> <td>1402</td> <td>330</td> <td>2402</td> <td>165</td> <td>Filing a brief in support of an appeal</td> <td></td> </tr> <tr> <td>1403</td> <td>290</td> <td>2403</td> <td>145</td> <td>Request for oral hearing</td> <td></td> </tr> <tr> <td>1451</td> <td>1,510</td> <td>1451</td> <td>1,510</td> <td>Petition to institute a public use proceeding</td> <td></td> </tr> <tr> <td>1452</td> <td>110</td> <td>2452</td> <td>55</td> <td>Petition to revive - unavoidable</td> <td></td> </tr> <tr> <td>1453</td> <td>1,330</td> <td>2453</td> <td>665</td> <td>Petition to revive - unintentional</td> <td></td> </tr> <tr> <td>1501</td> <td>1,330</td> <td>2501</td> <td>665</td> <td>Utility issue fee (or reissue)</td> <td></td> </tr> <tr> <td>1502</td> <td>480</td> <td>2502</td> <td>240</td> <td>Design issue fee</td> <td></td> </tr> <tr> <td>1503</td> <td>640</td> <td>2503</td> <td>320</td> <td>Plant issue fee</td> <td></td> </tr> <tr> <td>1460</td> <td>130</td> <td>1460</td> <td>130</td> <td>Petitions to the Commissioner</td> <td></td> </tr> <tr> <td>1807</td> <td>50</td> <td>1807</td> <td>50</td> <td>Processing fee under 37 CFR 1.17(q)</td> <td></td> </tr> <tr> <td>1806</td> <td>180</td> <td>1806</td> <td>180</td> <td>Submission of Information Disclosure Stmt</td> <td></td> </tr> <tr> <td>8021</td> <td>40</td> <td>8021</td> <td>40</td> <td>Recording each patent assignment per property (times number of properties)</td> <td></td> </tr> <tr> <td>1809</td> <td>770</td> <td>2809</td> <td>385</td> <td>Filing a submission after final rejection (37 CFR 1.129(a))</td> <td></td> </tr> <tr> <td>1810</td> <td>770</td> <td>2810</td> <td>385</td> <td>For each additional invention to be examined (37 CFR 1.129(b))</td> <td></td> </tr> <tr> <td>1801</td> <td>770</td> <td>2801</td> <td>385</td> <td>Request for Continued Examination (RCE)</td> <td></td> </tr> <tr> <td>1802</td> <td>900</td> <td>1802</td> <td>900</td> <td>Request for expedited examination of a design application</td> <td></td> </tr> <tr> <td colspan="5">Other fee (specify) _____</td> <td></td> </tr> <tr> <td colspan="5" style="text-align: right;"><b>* Reduced by Basic Filing Fee Paid</b></td> <td></td> </tr> <tr> <td colspan="5" style="text-align: right;"><b>SUBTOTAL (3)</b></td> <td style="border: 1px solid black; text-align: right;">(\$) 0</td> </tr> </tbody> </table>	Large Fee Code	Entity Fee (\$)	Small Fee Code	Entity Fee (\$)	Fee Description	Fee Paid	1051	130	2051	65	Surcharge - late filing fee or oath		1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet		1053	130	1053	130	Non-English specification		1812	2,520	1812	2,520	For filing a request for <i>ex parte</i> reexamination		1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action		1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action		1251	110	2251	55	Extension for reply within first month		1252	420	2252	210	Extension for reply within second month		1253	950	2253	475	Extension for reply within third month		1254	1,480	2254	740	Extension for reply within fourth month		1255	2,010	2255	1,005	Extension for reply within fifth month		1401	330	2401	165	Notice of Appeal		1402	330	2402	165	Filing a brief in support of an appeal		1403	290	2403	145	Request for oral hearing		1451	1,510	1451	1,510	Petition to institute a public use proceeding		1452	110	2452	55	Petition to revive - unavoidable		1453	1,330	2453	665	Petition to revive - unintentional		1501	1,330	2501	665	Utility issue fee (or reissue)		1502	480	2502	240	Design issue fee		1503	640	2503	320	Plant issue fee		1460	130	1460	130	Petitions to the Commissioner		1807	50	1807	50	Processing fee under 37 CFR 1.17(q)		1806	180	1806	180	Submission of Information Disclosure Stmt		8021	40	8021	40	Recording each patent assignment per property (times number of properties)		1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))		1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))		1801	770	2801	385	Request for Continued Examination (RCE)		1802	900	1802	900	Request for expedited examination of a design application		Other fee (specify) _____						<b>* Reduced by Basic Filing Fee Paid</b>						<b>SUBTOTAL (3)</b>					(\$) 0
Large Fee Code	Entity Fee (\$)	Small Fee Code	Entity Fee (\$)	Fee Description	Fee Paid																																																																																																																																																																																																																																																																																					
1001	770	2001	385	Utility filing fee																																																																																																																																																																																																																																																																																						
1002	340	2002	170	Design filing fee																																																																																																																																																																																																																																																																																						
1003	530	2003	265	Plant filing fee																																																																																																																																																																																																																																																																																						
1004	770	2004	385	Reissue filing fee																																																																																																																																																																																																																																																																																						
1005	160	2005	80	Provisional filing fee	80																																																																																																																																																																																																																																																																																					
<b>SUBTOTAL (1)</b>					(\$) 80																																																																																																																																																																																																																																																																																					
Total Claims	Extra Claims	Fee from below	Fee Paid																																																																																																																																																																																																																																																																																							
Independent Claims	-20** = 0	x	0																																																																																																																																																																																																																																																																																							
Multiple Dependent	-3** = 0	x	0																																																																																																																																																																																																																																																																																							
Large Fee Code	Entity Fee (\$)	Small Fee Code	Entity Fee (\$)	Fee Description																																																																																																																																																																																																																																																																																						
1202	18	2202	9	Claims in excess of 20																																																																																																																																																																																																																																																																																						
1201	86	2201	43	Independent claims in excess of 3																																																																																																																																																																																																																																																																																						
1203	290	2203	145	Multiple dependent claim, if not paid																																																																																																																																																																																																																																																																																						
1204	86	2204	43	**Reissue independent claims over original patent																																																																																																																																																																																																																																																																																						
1205	18	2205	9	**Reissue claims in excess of 20 and over original patent																																																																																																																																																																																																																																																																																						
<b>SUBTOTAL (2)</b>																																																																																																																																																																																																																																																																																										
Large Fee Code	Entity Fee (\$)	Small Fee Code	Entity Fee (\$)	Fee Description	Fee Paid																																																																																																																																																																																																																																																																																					
1051	130	2051	65	Surcharge - late filing fee or oath																																																																																																																																																																																																																																																																																						
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet																																																																																																																																																																																																																																																																																						
1053	130	1053	130	Non-English specification																																																																																																																																																																																																																																																																																						
1812	2,520	1812	2,520	For filing a request for <i>ex parte</i> reexamination																																																																																																																																																																																																																																																																																						
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action																																																																																																																																																																																																																																																																																						
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action																																																																																																																																																																																																																																																																																						
1251	110	2251	55	Extension for reply within first month																																																																																																																																																																																																																																																																																						
1252	420	2252	210	Extension for reply within second month																																																																																																																																																																																																																																																																																						
1253	950	2253	475	Extension for reply within third month																																																																																																																																																																																																																																																																																						
1254	1,480	2254	740	Extension for reply within fourth month																																																																																																																																																																																																																																																																																						
1255	2,010	2255	1,005	Extension for reply within fifth month																																																																																																																																																																																																																																																																																						
1401	330	2401	165	Notice of Appeal																																																																																																																																																																																																																																																																																						
1402	330	2402	165	Filing a brief in support of an appeal																																																																																																																																																																																																																																																																																						
1403	290	2403	145	Request for oral hearing																																																																																																																																																																																																																																																																																						
1451	1,510	1451	1,510	Petition to institute a public use proceeding																																																																																																																																																																																																																																																																																						
1452	110	2452	55	Petition to revive - unavoidable																																																																																																																																																																																																																																																																																						
1453	1,330	2453	665	Petition to revive - unintentional																																																																																																																																																																																																																																																																																						
1501	1,330	2501	665	Utility issue fee (or reissue)																																																																																																																																																																																																																																																																																						
1502	480	2502	240	Design issue fee																																																																																																																																																																																																																																																																																						
1503	640	2503	320	Plant issue fee																																																																																																																																																																																																																																																																																						
1460	130	1460	130	Petitions to the Commissioner																																																																																																																																																																																																																																																																																						
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)																																																																																																																																																																																																																																																																																						
1806	180	1806	180	Submission of Information Disclosure Stmt																																																																																																																																																																																																																																																																																						
8021	40	8021	40	Recording each patent assignment per property (times number of properties)																																																																																																																																																																																																																																																																																						
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))																																																																																																																																																																																																																																																																																						
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))																																																																																																																																																																																																																																																																																						
1801	770	2801	385	Request for Continued Examination (RCE)																																																																																																																																																																																																																																																																																						
1802	900	1802	900	Request for expedited examination of a design application																																																																																																																																																																																																																																																																																						
Other fee (specify) _____																																																																																																																																																																																																																																																																																										
<b>* Reduced by Basic Filing Fee Paid</b>																																																																																																																																																																																																																																																																																										
<b>SUBTOTAL (3)</b>					(\$) 0																																																																																																																																																																																																																																																																																					

<b>SUBMITTED BY</b>		<b>Complete (if applicable)</b>	
Name (Print/Type)	Paul M. Buch	Registration No. (Attorney/Agent)	40,244
Signature		Date	August 6, 2004
		Telephone	202-912-2000
		Customer No. 26633	

Attorney Docket No. 37629-0085PVC

**Inventor: Michael Tesar**

**Residence: Weilheim, German**

**Citizenship: German**

**Anti-CD38 Human Antibodies**

**and**

**Uses Therefor**

### BACKGROUND OF THE INVENTION

CD38 is a type-II membrane glycoprotein and belongs to the family of ectoenzymes, due to its enzymatic activity as ADP ribosyl-cyclase and cADP-hydrolase. During ontogeny, CD38 appears on CD34<sup>+</sup> committed stem cells and lineage-committed progenitors of lymphoid, erythroid and myeloid cells. It is understood that CD38 expression persists only in the lymphoid lineage, through the early stages of T- and B-cell development.

The up-regulation of CD38 serves as a marker for lymphocyte activation—in particular B-cell differentiation along the plasmacytoid pathway. (Co-)receptor functions of CD38 leading to intracellular signaling or intercellular communication via its ligand, CD31, are postulated, as well as its role as an intracellular regulator of a second messenger, cyclic ADPr, in a variety of signaling cascades. However, its physiological importance remains to be elucidated, since knock out of the murine analogue or anti-CD38 auto-antibodies in humans do not appear to be detrimental.

Apart from its expression in the hematopoietic system, researchers have noted the up-regulation of CD38 on various cell-lines derived from B-, T-, and myeloid/monocytic tumors, including B- or T-cell acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), Non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM). In MM, for example, strong CD38 expression is witnessed in the majority of all patient samples.

Hence, over-expression of CD38 on malignant cells provides an attractive therapeutic target for immunotherapy. Of special attraction is the fact that the most primitive pluripotent stem cells of the hematopoietic system are CD38-negative and that the extent of cytotoxic effects by ADCC or CDC correlates well with the expression-levels of the respective target.

Current approaches of anti-CD38 therapies can be divided in two groups: in vivo and ex vivo approaches. In in vivo approaches, anti-CD38 antibodies are administered to a subject in need of therapy in order to cause the antibody-mediated depletion of CD38-overexpressing malignant cells. Depletion can either  
5 be achieved by antibody-mediated ADCC and/or CDC by effector cells, or by using the anti-CD38 antibodies as targeting moieties for the transport of cytotoxic substances, e.g. saporin, to the target cells, and subsequent internalization. In the ex vivo approach, cell population, e.g. bone marrow cells, comprising CD38 overexpressing malignant cells are removed from an individual in need of  
10 treatment and are contacted with anti-CD38 antibodies. The target cells are either destroyed by cytotoxic substances, e.g. saporin, as described for the in vivo approach, or are removed by contacting the cell population with immobilized anti-CD38 antibodies, thus removing CD38 overexpressing target cells from the mixture. Thereafter, the depleted cell population is reinserted into the patient.

15 Antibodies specific for CD38 can be divided in different groups, depending on various properties. Binding of some antibodies to the CD38 molecule (predominantly aa 220-300) can trigger activities within the target cell, such as Ca<sup>2+</sup> release, cytokine release, phosphorylation events and growth stimulation based on the respective antibody specificity (Konopleva et al., 1998; Ausiello et  
20 al., 2000), but no clear correlation between the binding site of the various known antibodies and their (non-)agonistic properties could be seen (Funaro et al., 1990). However, all antibodies seem to exclusively recognize epitopes (amino acid residues 220 to 300) located in the C-terminal part of CD38. No antibodies are known so far, which would be specific for epitopes in the N-terminal part of  
25 CD38 far distant from the active site in the primary protein sequence.

Relatively little is known about the efficacy of published anti-CD38 antibodies. However, we have found that OKT10, which has been in clinical testing, has a relatively low affinity and efficacy when analyzed as chimeric construct comprising a human Fc part. Furthermore, OKT10 is a murine  
5 antibody, so that any chimeric or humanized OKT10-based antibody construct would still comprise sequences of murine origin in at least the variable domains.

A human anti-CD38 scFv antibody fragment has recently been described (WO 02/06347). However, that antibody is specific for a subset of CD38 molecules only.

10 Correspondingly, in light of the great potential for anti-CD38 antibody therapy, there is a high need for human anti-CD38 antibodies with high affinity and with high efficacy in mediating killing of CD38 overexpressing malignant cells by ADCC and/or CDC.

The present invention satisfies these and other needs by providing fully  
15 human and highly efficacious anti-CD38 antibodies, which are described below.

#### SUMMARY OF THE INVENTION

It is an object of the invention to provide human antibodies that can effectively mediate the killing of CD38-overexpressing cells.

20 It is another object of the invention to provide antibodies that are safe for human administration.

It is also an object of the present invention to provide methods for treating disease or and/or conditions associated with CD38 up-regulation by using one or more antibodies of the invention. These and other objects of the invention are  
25 more fully described herein.



In one aspect, the invention provides an isolated human antibody or functional antibody fragment that contains an antigen-binding region that is specific for an epitope of CD38, where the human antibody or functional fragment thereof is able to mediate killing of a CD38+ target cell (LP-1 (DSMZ: ACC41) and RPMI-8226 (ATCC: CCL-155)) by antibody-dependent cellular cytotoxicity ("ADCC") with an at least two- to five-fold better efficacy than the chimeric OKT10 antibody having SEQ ID NOS: 23 and 24 (under the same or substantially the same conditions), when a human PBMC cell is employed as an effector cell, and when the ratio of effector cells to target cells is between about 30:1 and about 50:1. Such an antibody or functional fragment thereof may contain an antigen-binding region that contains an H-CDR3 region depicted in SEQ ID NO: 5, 6, 7, or 8; the antigen-binding region may further include an H-CDR2 region depicted in SEQ ID NO: 5, 6, 7, or 8; and the antigen-binding region also may contain an H-CDR1 region depicted in SEQ ID NO: 5, 6, 7, or 8. Such a CD38-specific antibody of the invention may contain an antigen-binding region that contains an L-CDR3 region depicted in SEQ ID NO: 13, 14, 15, or 16; the antigen-binding region may further include an L-CDR1 region depicted in SEQ ID NO: 13, 14, 15, or 16; and the antigen-binding region also may contain an L-CDR2 region depicted in SEQ ID NO: 13, 14, 15, or 16.

In another aspect, the invention provides an isolated human antibody or functional antibody fragment that contains an antigen-binding region that is specific for an epitope of CD38, where the human antibody or functional fragment thereof is able to mediate killing of a CD38-transfected CHO cell by CDC with an at least two-fold better efficacy than chimeric OKT10 (SEQ ID NOS: 23 and 24) under the same or substantially the same conditions. An antibody satisfying these criteria may contain an antigen-binding region that contains an H-CDR3 region

depicted in SEQ ID NO: 5, 6, or 7; the antigen-binding region may further include an H-CDR2 region depicted in SEQ ID NO: 5, 6, or 7; and the antigen-binding region also may contain an H-CDR1 region depicted in SEQ ID NO: 5, 6, or 7. Such a CD38-specific antibody of the invention may contain an antigen-binding  
5 region that contains an L-CDR3 region depicted in SEQ ID NO: 13, 14, or 15; the antigen-binding region may further include an L-CDR1 region depicted in SEQ ID NO: 13, 14, or 15; and the antigen-binding region also may contain an L-CDR2 region depicted in SEQ ID NO: 13, 14, or 15.

Antibodies (and functional fragments thereof) of the invention may  
10 contain an antigen-binding region that is specific for an epitope of CD38, which epitope contains one or more amino acid residues of amino acid residues 43 to 215 of CD38 as depicted by SEQ ID NO: 22. More specifically, an epitope to which the antigen-binding region binds may contain one or more amino acid residues found in one or more of the amino acid stretches taken from the list of  
15 amino acid stretches 44-66, 82-94, 142-154, 148-164, 158-170, and 192-206. For certain antibodies, the epitope may be linear, whereas for others, it may be conformational (*i.e.*, discontinuous). An antibody or functional fragment thereof having one or more of these properties may contain an antigen-binding region that contains an H-CDR3 region depicted in SEQ ID NO: 5, 6, 7, or 8; the antigen-  
20 binding region may further include an H-CDR2 region depicted in SEQ ID NO: 5, 6, 7, or 8; and the antigen-binding region also may contain an H-CDR1 region depicted in SEQ ID NO: 5, 6, 7, or 8. Such a CD38-specific antibody of the invention may contain an antigen-binding region that contains an L-CDR3 region depicted in SEQ ID NO: 13, 14, 15, or 16; the antigen-binding region may further  
25 include an L-CDR1 region depicted in SEQ ID NO: 13, 14, 15, or 16; and the

antigen-binding region also may contain an L-CDR2 region depicted in SEQ ID NO: 13, 14, 15, or 16.

Peptide variants of the sequences disclosed herein are also embraced by the present invention. Accordingly, the invention includes human anti-CD38  
5 antibodies having a heavy chain amino acid sequence with: at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 6, 7, or 8; and/or at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 6, 7, or 8. Further included are human anti-CD38 antibodies having a light chain amino acid  
10 sequence with: at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 14, 15 or 16; and/or at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 14, 15 or 16.

An antibody of the invention may be an IgG (*e.g.*, IgG<sub>1</sub>), while an  
15 antibody fragment may be a Fab or scFv, for example. An inventive antibody fragment, accordingly, may be, or may contain, an antigen-binding region that behaves in one or more ways as described herein.

The invention also is related to isolated nucleic acid sequences, each of which can encode an antigen-binding region of a human antibody or functional  
20 fragment thereof that is specific for an epitope of CD38. Such a nucleic acid sequence may encode a variable heavy chain of an antibody and include a sequence selected from the group consisting of SEQ ID NOS: 1, 2, 3, or 4, or a nucleic acid sequence that hybridizes under high stringency conditions to the complementary strand of SEQ ID NO: 1, 2, 3, or 4. The nucleic acid might  
25 encode a variable light chain of an isolated antibody or functional fragment thereof, and may contain a sequence selected from the group consisting of SEQ

ID NOS: 9, 10, 11, or 12, or a nucleic acid sequence that hybridizes under high stringency conditions to the complementary strand of SEQ ID NO: 9, 10, 11, or 12.

Nucleic acids of the invention are suitable for recombinant production.

5 Thus, the invention also relates to vectors and host cells containing a nucleic acid sequence of the invention.

Compositions of the invention may be used for therapeutic or prophylactic applications. The invention, therefore, includes a pharmaceutical composition containing an inventive antibody (or functional antibody fragment) and a  
10 pharmaceutically acceptable carrier or excipient therefor. In a related aspect, the invention provides a method for treating a disorder or condition associated with the undesired presence of CD38 or CD38 expressing cells. Such method contains the steps of administering to a subject in need thereof an effective amount of the pharmaceutical composition that contains an inventive antibody as described or  
15 contemplated herein.

### **BRIEF DESCRIPTION OF THE FIGURES**

Figure 1a provides nucleic acid sequences of various novel antibody  
20 variable heavy regions.

Figure 1b provides amino acid sequences of various novel antibody variable heavy regions. CDR regions HCDR1, HCDR2 and HCDR3 are designated from N- to C-terminus in boldface.

Figure 2a provides nucleic acid sequences of various novel antibody  
25 variable light regions.

Figure 2b provides amino acid sequences of various novel antibody variable light regions. CDR regions LCDR1, LCDR2 and LCDR3 are designated from N- to C-terminus in boldface.

Figure 3 provides amino acid sequences of variable heavy regions of various consensus-based HuCAL antibody master gene sequences. CDR regions HCDR1, HCDR2 and HCDR3 are designated from N- to C-terminus in boldface.

Figure 4 provides amino acid sequences of variable light regions of various consensus-based HuCAL antibody master gene sequences. CDR regions LCDR1, LCDR2 and LCDR3 are designated from N- to C-terminus in boldface.

Figure 5 provides the amino acid sequence of CD38 (SWISS-PROT primary accession number P28907).

Figure 6 provides the nucleotide sequences of the heavy and light chains of chimeric OKT10.

Figure 7 provides a schematic overview of epitopes of representative antibodies of the present invention.

Figure 8 provides the DNA sequence of pMORPH®\_h\_IgG1\_1 (bp 601-2100) (SEQ ID NO: 32): The vector is based on the pcDNA3.1+ vectors (Invitrogen). The amino acid sequence of the VH-stuffer sequence is indicated in bold, whereas the final reading frames of the VH-leader sequence and the constant region gene are printed in non-bold. Restriction sites are indicated above the sequence. The priming sites of the sequencing primers are underlined.

Figure 9 provides the DNA sequence of Ig kappa light chain expression vector pMORPH®\_h\_Igκ\_1 (bp 601-1400) (SEQ ID NO: 33): The vector is based on the pcDNA3.1+ vectors (Invitrogen). The amino acid sequences of the Vκ-stuffer sequence is indicated in bold, whereas the final reading frames of the

V $\kappa$ -leader sequence and of the constant region gene are printed in non-bold.

Restriction sites are indicated above the sequence. The priming sites of the sequencing primers are underlined.

Figure 10 provides the DNA sequence of HuCAL Ig lambda light chain  
5 vector pMORPH@\_h\_Ig $\lambda$ \_1 (bp 601-1400) (SEQ ID NO: 34): The amino acid  
sequence of the V $\lambda$ -stuffer sequence is indicated in bold, whereas the final  
reading frames of the V $\lambda$ -leader sequence and of the constant region gene are  
printed in non-bold. Restriction sites are indicated above the sequence. The  
priming sites of the sequencing primers are underlined.

10 Figure 11 provides the results of the proliferation assay: PBMCs from 6  
different healthy donors (as indicated by individual dots) were cultured for 3 days  
in the presence of HuCAL® antibodies Mab#1 (=MOR03077), Mab#2  
(=MOR03079), and Mab#3 (=MOR03080), the reference antibody chOKT10, the  
agonistic (ag.) control IB4, an irrelevant HuCAL® negative control IgG1 (NC)  
15 and a murine IgG2a (Iso) as matched isotype control for IB4. A standard labeling  
with BrdU was used to measure proliferation activity and its incorporation (as  
RLU = relative light units) analyzed via a chemiluminescence-based ELISA.

Figure 12 provides the results of the IL-6 Release Assay: PBMCs from 4-8  
different healthy donors (as indicated by individual dots) were cultured for 24 hrs  
20 in the presence of HuCAL® antibodies Mab#1 (=MOR03077), Mab#2  
(=MOR03079), and Mab#3 (=MOR03080), the reference antibody chOKT10, the  
agonistic (ag.) control IB4, an irrelevant HuCAL® negative control (NC) and  
medium only (Medium). IL-6 content in relative light units (RLU) was analyzed  
from culture supernatants via a chemiluminescence based ELISA.

Figure 13 provides data about the cytotoxicity towards CD34+/CD38+ progenitor cells: PBMCs from healthy donors harboring autologous CD34+/CD38+ progenitor cells were incubated with HuCAL® Mab#1 (=MOR03077), Mab#2 (=MOR03079), and Mab#3 (=MOR03080), the positive control (PC = chOKT10) and an irrelevant HuCAL® negative control for 4 hours, respectively. Afterwards, the cell suspension was mixed with conditioned methyl-cellulose medium and incubated for 2 weeks. Colony forming units (CFU) derived from erythroid burst forming units (BFU-E; panel B) and granulocyte/erythroid/macrophage/ megakaryocyte stem cells (CFU-GEMM; panels B) and granulocyte/macrophage stem cells (CFU-GM; panel C) were counted and normalized against the medium control ("none" = medium). Panel A represents the total number of CFU (Total CFUc) for all progenitors. Mean values from at least 10 different PBMC donors are given. Error bars represent standard error of the mean.

Figure 14 provides data about ADCC with different cell-lines:

a: Single measurements (except for RPMI8226: average from 4 indiv. Assays); E:T -ratio: 30:1

b: Namba et al., 1989

c: 5 µg/ml used for antibody conc. (except for Raji with 0.1 µg/ml)

d: addition of retinoic assay for stimulation of CD38-expression  
specific killing [%] = [(exp. killing - medium killing) / (1 - medium killing)] \* 100

PC: Positive control (=chOKT10)

MM: Multiple myeloma

CLL: Chronic B-cell leukemia

ALL: Acute lymphoblastic leukemia

AML: Acute myeloid leukemia

DSMZ: Deutsche Sammlung für Mikroorganismen und  
Zellkulturen GmbH

5 ATCC: American type culture collection

ECACC: European collection of cell cultures

MFI: Mean fluorescence intensities.

Figure 15 provides data about ADCC with MM-samples:

<sup>a</sup>: 2-4 individual analyses

10

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the discovery of novel human antibodies that are specific to or have a high affinity for CD38 and can deliver a therapeutic benefit to a subject. The antibodies of the invention can be used in many  
15 contexts, which are more fully described herein.

A “human” antibody or functional antibody fragment is hereby defined as one that not chimeric (e.g., not “humanized”) and not from (either in whole or in part) a non-human species. A human antibody or functional antibody fragment can be derived directly from a human or can be derived at least partially *in silico*  
20 from synthetic sequences that are based on the analysis of known human antibody sequences.

As used herein, an antibody “binds specifically to,” is “specific to/for” or “specifically recognizes” an antigen (here, CD38) if such antibody is able to discriminate between such antigen and one or more reference antigen(s), since  
25 binding specificity is not an absolute, but a relative property. In its most general



form (and when no defined reference is mentioned), “specific binding” is referring to the ability of the antibody to discriminate between the antigen of interest and an unrelated antigen, as determined, for example, in accordance with one of the following methods. Such methods comprise, but are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-tests and peptide scans. For example, a standard ELISA assay can be carried out. The scoring may be carried out by standard color development (e.g. secondary antibody with horseradish peroxidase and tetramethyl benzidine with hydrogenperoxide). The reaction in certain wells is scored by the optical density, for example, at 450 nm. Typical background (=negative reaction) may be 0.1 OD; typical positive reaction may be 1 OD. This means the difference positive/negative can be more than 10-fold. Typically, determination of binding specificity is performed by using not a single reference antigen, but a set of about three to five unrelated antigens, such as milk powder, BSA, transferrin or the like.

However, “specific binding” also may refer to the ability of an antibody to discriminate between the target antigen and one or more closely related antigen(s), which are used as reference points, e.g. between CD38 and CD157. Additionally, “specific binding” may relate to the ability of an antibody to discriminate between different parts of its target antigen, e.g. different domains or regions of CD38, such as epitopes in the N-terminal or in the C-terminal region of CD38, or between one or more key amino acid residues or stretches of amino acid residues of CD38.

Also, as used herein, an “immunoglobulin” (Ig) hereby is defined as a protein belonging to the class IgG, IgM, IgE, IgA, or IgD (or any subclass thereof), and includes all conventionally known antibodies and functional fragments thereof. A “functional fragment” of an antibody/immunoglobulin hereby is defined as a fragment of an antibody/immunoglobulin (e.g., a variable

region of an IgG) that retains the antigen-binding region. An “antigen-binding region” of an antibody typically is found in one or more hypervariable region(s) of an antibody, *i.e.*, the CDR-1, -2, and/or -3 regions; however, the variable “framework” regions can also play an important role in antigen binding, such as

5 by providing a scaffold for the CDRs. Preferably, the “antigen-binding region” comprises at least amino acid residues 4 to 103 of the variable light (VL) chain and 5 to 109 of the variable heavy (VH) chain, more preferably amino acid residues 3 to 107 of VL and 4 to 111 of VH, and particularly preferred are the complete VL and VH chains (amino acid positions 1 to 109 of VL and 1 to 113 of

10 VH; numbering according to WO 97/08320). A preferred class of immunoglobulins for use in the present invention is IgG. “Functional fragments” of the invention include the domain of a F(ab')<sub>2</sub> fragment, a Fab fragment and scFv. The F(ab')<sub>2</sub> or Fab may be engineered to minimize or completely remove the intermolecular disulphide interactions that occur between the C<sub>H1</sub> and C<sub>L</sub>

15 domains.

An antibody of the invention may be derived from a recombinant antibody library that is based on amino acid sequences that have been designed *in silico* and encoded by nucleic acids that are synthetically created. *In silico* design of an antibody sequence is achieved, for example, by analyzing a database of human

20 sequences and devising a polypeptide sequence utilizing the data obtained therefrom. Methods for designing and obtaining *in silico*-created sequences are described, for example, in Knappik *et al.*, J. Mol. Biol. (2000) 296:57; Krebs *et al.*, J. Immunol. Methods. (2001) 254:67; and U.S. Patent No. 6,300,064 issued to Knappik *et al.*, which hereby are incorporated by reference in their entirety.

**Antibodies of the Invention**

Throughout this document, reference is made to the following representative antibodies of the invention: "antibody nos." or "LACS" or "MOR" 3077, 3079, 3080 and 3100. LAC 3077 represents an antibody having a variable heavy region  
5 corresponding to SEQ ID NO: 1 (DNA)/SEQ ID NO: 5 (protein) and a variable light region corresponding to SEQ ID NO: 9 (DNA)/SEQ ID NO: 13 (protein). LAC 3079 represents an antibody having a variable heavy region corresponding to SEQ ID NO: 2 (DNA)/SEQ ID NO: 6 (protein) and a variable light region corresponding to SEQ ID NO: 10 (DNA)/SEQ ID NO: 14 (protein). LAC 3080  
10 represents an antibody having a variable heavy region corresponding to SEQ ID NO: 3 (DNA)/SEQ ID NO: 7 (protein) and a variable light region corresponding to SEQ ID NO: 11 (DNA)/SEQ ID NO: 15 (protein). LAC 3100 represents an antibody having a variable heavy region corresponding to SEQ ID NO: 4 (DNA)/SEQ ID NO: 8 (protein) and a variable light region corresponding to SEQ  
15 ID NO: 12 (DNA)/SEQ ID NO: 16 (protein).

In one aspect, the invention provides antibodies having an antigen-binding region that can bind specifically to or has a high affinity for one or more regions of CD38, whose amino acid sequence is depicted by SEQ ID NO: 22. An antibody is said to have a "high affinity" for an antigen if the affinity  
20 measurement is at least 100 nM (monovalent affinity of Fab fragment). An inventive antibody or antigen-binding region preferably can bind to CD38 with an affinity of about less than 100 nM, more preferably less than about 60 nM, and still more preferably less than about 30 nM. Further preferred are antibodies that bind to CD38 with an affinity of less than about 10 nM, and more preferably less  
25 than 3 about nM. For instance, the affinity of an antibody of the invention against CD38 may be about 10.0 nM or 2.4 nM (monovalent affinity of Fab fragment).

Table 1 provides a summary of affinities of representative antibodies of the invention, as determined by surface plasmon resonance (Biacore) and FACS Scatchard analysis:

5           **Table 1: Antibody Affinities**

Antibody (Fab or IgG1)	BIACORE (Fab) $K_D$ [nM] <sup>a</sup>	FACS Scatchard (IgG1) <sup>b</sup> $K_D$ [nM] <sup>a</sup>
MOR03077	56.0	0.89
MOR03079	2.4	0.60
MOR03080	27.5	0.47
MOR03100	10.0	6.31
Chimeric OKT10	not determined	8.28

<sup>a</sup>: mean from at least 2 different affinity determinations

<sup>b</sup>: RPMI8226 MM cell-line used for FACS-Scatchards

10           With reference to Table 1, the affinity of LACs 3077, 3079, 3080 and 3100 was measured by surface plasmon resonance (Biacore) on immobilized recombinant CD38 and by a flow cytometry procedure utilizing the CD38-expressing human RPMI8226 cell line. The Biacore studies were performed on directly immobilized antigen (CD38-Fc fusion protein). The Fab format of LACs  
15 3077, 3079, 3080 and 3100 exhibit an monovalent affinity range between about 2.4 and 56 nM on immobilized CD38-Fc fusion protein with LAC 3079 showing the highest affinity, followed by Fabs 3100, 3080 and 3077.

The IgG1 format was used for the cell-based affinity determination (FACS Scatchard). The right column of Table 1 denotes the binding strength of the LACS in this format. LAC 3080 showed the strongest binding, which is slightly stronger than LACS 3079 and 3077.

5 Another feature of preferred antibodies of the invention is their specificity for an area within the N-terminal region of CD38. All representative LACS 3077, 3079, 3080, and 3100 of the invention can bind specifically to the N-terminal region of CD38.

The type of epitope to which an antibody of the invention binds may be  
10 linear (i.e. one consecutive stretch of amino acids) or conformational (i.e. multiple stretches of amino acids). In order to determine whether the epitope of a particular antibody is linear or conformational, the skilled worker can analyze the binding of antibodies to overlapping peptides (*e.g.*, 13-mer peptides with an overlap of 11 amino acids) covering different domains of CD38. Using this  
15 analysis, the inventors have discovered that LACS 3077, 3080, and 3100 recognize discontinuous epitopes in the N-terminal region of CD38, whereas the epitope of LAC 3079 can be described as linear (see Figure 7).

An antibody of the invention preferably is species cross-reactive with humans and at least one other species, which may be a rodent species or a non-  
20 human primate. The non-human primate can be rhesus, baboon and/or cynomolgus. The rodent species can be mouse, rat and/or hamster. An antibody that is cross reactive with at least one rodent species, for example, can provide greater flexibility and benefits over known anti-CD38 antibodies, for purposes of conducting *in vivo* studies in multiple species with the same antibody.

Preferably, an antibody of the invention not only is able to bind to CD38, but also is able to mediate killing of a cell expressing CD38. More specifically, an antibody of the invention can mediate its therapeutic effect by depleting CD38-positive (*e.g.*, malignant) cells via antibody-effector functions. These functions include antibody-dependent cellular cytotoxicity (ADCC) and complement mediated cytotoxicity (CDC).

Table 2 provides a summary of the determination of EC50 values of representative antibodies of the invention in both ADCC and CDC:

10           **Table 2: EC50 Values of Antibodies**

Antibody (IgG1)	ADCC		CDC
	EC50 [nM]		EC50 [nM]
	LP-1	RPMI8226	CHO-transfectants
<b>MOR03077</b>	0.60 <sup>a</sup>	0.08 <sup>a</sup>	0.8 <sup>c</sup> ; 0.94 <sup>d</sup>
<b>MOR03079</b>	0.09 <sup>a</sup>	0.04 <sup>a</sup>	0.41 <sup>c</sup>
<b>MOR03080</b>	0.17 <sup>b</sup>	0.05 <sup>a</sup>	3.2 <sup>c</sup> ; 2.93 <sup>d</sup>
<b>MOR03100</b>	1.00 <sup>b</sup>	0.28 <sup>a</sup>	10.9 <sup>c</sup> ; 13.61 <sup>c</sup>
<b>Chimeric OKT10</b>	5.23 <sup>a</sup>	4.10 <sup>a</sup>	9.30 <sup>c</sup>

<sup>a</sup>: mean from at least 2 EC50 determinations

<sup>b</sup>: single determination

<sup>c</sup>: mean from 2 EC50 determinations

<sup>d</sup>: mean from 3 EC50 determinations

15           <sup>e</sup>: mean from 4 EC50 determinations

CD38-expression, however, is not only found on immune cells within the myeloid (e.g. monocytes, granulocytes) and lymphoid lineage (e.g. activated B and T-cells; plasma cells), but also on the respective precursor cells. Since it is  
5 important that those cells are not affected by antibody-mediated killing of malignant cells, the antibodies of the present invention are preferably not cytotoxic to precursor cells.

In addition to its catalytic activities as a cyclic ADP-ribose cyclase and  
10 hydrolase, CD38 displays the ability to transduce signals of biological relevance (Hoshino *et al.*, 1997; Ausiello *et al.*, 2000). Those functions can be induced *in vivo* by e.g. receptor-ligand interactions or by cross-linking with agonistic anti-CD38 antibodies, leading e.g. to calcium mobilization, lymphocyte proliferation and release of cytokines. Preferably, the antibodies of the present invention are  
15 non-agonistic antibodies.

### Peptide Variants

Antibodies of the invention are not limited to the specific peptide sequences  
20 provided herein. Rather, the invention also embodies variants of these polypeptides. With reference to the instant disclosure and conventionally available technologies and references, the skilled worker will be able to prepare, test and utilize functional variants of the antibodies disclosed herein, while appreciating that variants having the ability to mediate killing of a CD38+ target  
25 cell fall within the scope of the present invention. As used in this context, “ability to mediate killing of a CD38+ target cell” means a functional characteristic

ascribed to an anti-CD38 antibody of the invention. Ability to mediate killing of a CD38+ target cell, thus, includes the ability to mediate killing of a CD38+ target cell, *e.g.*, by ADCC and/or CDC, or by toxin constructs conjugated to an antibody of the invention.

5           A variant can include, for example, an antibody that has at least one altered complementarity determining region (CDR) (hyper-variable) and/or framework (FR) (variable) domain/position, *vis-à-vis* a peptide sequence disclosed herein. To better illustrate this concept, a brief description of antibody structure follows.

          An antibody is composed of two peptide chains, each containing one (light  
10 chain) or three (heavy chain) constant domains and a variable region (VL, VH), the latter of which is in each case made up of four FR regions and three interspaced CDRs. The antigen-binding site is formed by one or more CDRs, yet the FR regions provide the structural framework for the CDRs and, hence, play an important role in antigen binding. By altering one or more amino acid residues in  
15 a CDR or FR region, the skilled worker routinely can generate mutated or diversified antibody sequences, which can be screened against the antigen, for new or improved properties, for example.

          Tables 3a (VH) and 3b (VL) delineate the CDR and FR regions for certain antibodies of the invention and compare amino acids at a given position to each  
20 other and to corresponding consensus or “master gene” sequences (as described in U.S. Patent No. 6,300,064):



### Table 3a: VH Sequences

[illegible]

### Table 3a: VH Sequences

[illegible]

Table 3b: VL Sequences

VL sequences CD38 binders																																			
Framework 1														Framework 2																					
CDR1														CDR2																					
3														5																					
1														4																					
2														6																					
Position	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	
	ECORV	SeqAI	BssSI	KpnI	XmaI	BbeI	Bsu36I	Bam																											
VL3	S	E	L	T	Q	P	P	-	S	V	S	V	A	P	G	Q	T	A	R	I	S	C	S	G	D	A	L	G	D	-	-	-	-	-	-
3080	D	I	E	L	T	Q	P	P	-	S	V	S	V	A	P	G	Q	T	A	R	I	S	C	S	G	D	N	I	G	N	-	-	-	-	-
3100	D	I	E	L	T	Q	P	P	-	S	V	S	V	A	P	G	Q	T	A	R	I	S	C	S	G	D	N	I	G	N	-	-	-	-	-
VLK1	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	
3079	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	G	I	S	-	-	-	-	-
VLK2	D	I	V	M	T	Q	S	P	L	S	L	P	V	T	P	G	E	P	A	S	I	S	C	R	S	S	Q	S	L	L	H	S	-	-	-
3077	D	I	V	M	T	Q	S	P	L	S	L	P	V	T	P	G	E	P	A	S	I	S	C	R	S	S	Q	S	L	L	H	S	-	-	-

Table 3b: VL Sequences

		VL																							
		CDR3												CDR4											
		Framework 3												Framework 4											
		1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Position		1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
VLJ3	SEQ ID NO 21	S	N	S	G	N	T	A	T	L	T	I	S	G	T	Q	A	E	D	E	A	D	Y	C	Q
3080	SEQ ID NO 13	S	N	S	G	N	T	A	T	L	T	I	S	G	T	Q	A	E	D	E	A	D	Y	C	S
3100	SEQ ID NO 15	S	N	S	G	N	T	A	T	L	T	I	S	G	T	Q	A	E	D	E	A	D	Y	C	S
VLK1	SEQ ID NO 22	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	C	Q
3079	SEQ ID NO 14	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	C	Q
VLK2	SEQ ID NO 22	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	C	Q
3077	SEQ ID NO 14	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	C	Q

The skilled worker can use the data in Tables 3a and 3b to design peptide variants that are within the scope of the present invention. It is preferred that variants are constructed by changing amino acids within one or more CDR regions; a variant might also have one or more altered framework regions. With  
5 reference to a comparison of the novel antibodies to each other, candidate residues that can be changed include e.g. residues 4 or 37 of the variable light and e.g. residues 13 or 43 of the variable heavy chains of LACs 3080 and 3077, since these are positions of variance vis-à-vis each other. Alterations also may be made in the framework regions. For example, a peptide FR domain might be altered  
10 where there is a deviation in a residue compared to a germline sequence.

With reference to a comparison of the novel antibodies to the corresponding consensus or "master gene" sequence, candidate residues that can be changed include e.g. residues 27, 50 or 90 of the variable light chain of LAC 3080 compared to VL $\lambda$ 3 and e.g. residues 33, 52 and 97 of the variable heavy chain of  
15 LAC 3080 compared to VH3. Alternatively, the skilled worker could make the same analysis by comparing the amino acid sequences disclosed herein to known sequences of the same class of such antibodies, using, for example, the procedure described by Knappik *et al.*, 2000 and U.S. Patent No. 6,300,064 issued to Knappik *et al.*

20 Furthermore, variants may be obtained by using one LAC as starting point for optimization by diversifying one or more amino acid residues in the LAC, preferably amino acid residues in one or more CDRs, and by screening the resulting collection of antibody variants for variants with improved properties. Particularly preferred is diversification of one or more amino acid residues in  
25 CDR-3 of VL, CDR-3 of VH, CDR-1 of VL and/or CDR-2 of VH. Diversification can be done by synthesizing a collection of DNA molecules using

trinucleotide mutagenesis (TRIM) technology (Virnekäs, B., Ge, L., Plückthun, A., Schneider, K.C., Wellnhofer, G., and Moroney S.E. (1994) Trinucleotide phosphoramidites: ideal reagents for the synthesis of mixed oligonucleotides for random mutagenesis. Nucl. Acids Res. 22, 5600.).

5    ***Conservative Amino Acid Variants***

Polypeptide variants may be made that conserve the overall molecular structure of an antibody peptide sequence described herein. Given the properties of the individual amino acids, some rational substitutions will be recognized by the skilled worker. Amino acid substitutions, *i.e.*, "conservative substitutions,"  
10    may be made, for instance, on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved.

For example, (a) nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; (b)  
15    polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; (c) positively charged (basic) amino acids include arginine, lysine, and histidine; and (d) negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Substitutions typically may be made within groups (a)-(d). In addition, glycine and proline may be substituted for one  
20    another based on their ability to disrupt  $\alpha$ -helices. Similarly, certain amino acids, such as alanine, cysteine, leucine, methionine, glutamic acid, glutamine, histidine and lysine are more commonly found in  $\alpha$ -helices, while valine, isoleucine, phenylalanine, tyrosine, tryptophan and threonine are more commonly found in  $\beta$ -pleated sheets. Glycine, serine, aspartic acid, asparagine, and proline are  
25    commonly found in turns. Some preferred substitutions may be made among the following groups: (i) S and T; (ii) P and G; and (iii) A, V, L and I. Given the

known genetic code, and recombinant and synthetic DNA techniques, the skilled scientist readily can construct DNAs encoding the conservative amino acid variants. In one particular example, amino acid position 3 in SEQ ID NOS: 5, 6, 7, and/or 8 can be changed from a Q to an E.

5       As used herein, "sequence identity" between two polypeptide sequences indicates the percentage of amino acids that are identical between the sequences. "Sequence similarity" indicates the percentage of amino acids that either are identical or that represent conservative amino acid substitutions. Preferred polypeptide sequences of the invention have a sequence identity in the CDR  
10 regions of at least 60%, more preferably, at least 70% or 80%, still more preferably at least 90% and most preferably at least 95%. Preferred antibodies also have a sequence similarity in the CDR regions of at least 80%, more preferably 90% and most preferably 95%.

#### **DNA molecules of the invention**

15       The present invention also relates to the DNA molecules that encode an antibody of the invention. These sequences include, but are not limited to, those DNA molecules set forth in Figures 1a and 2a.

DNA molecules of the invention are not limited to the sequences disclosed herein, but also include variants thereof. DNA variants within the invention may  
20 be described by reference to their physical properties in hybridization. The skilled worker will recognize that DNA can be used to identify its complement and, since DNA is double stranded, its equivalent or homolog, using nucleic acid hybridization techniques. It also will be recognized that hybridization can occur with less than 100% complementarity. However, given appropriate choice of  
25 conditions, hybridization techniques can be used to differentiate among DNA sequences based on their structural relatedness to a particular probe. For guidance

regarding such conditions see, Sambrook et al., 1989 (Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning: A laboratory manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, USA) and Ausubel et al., 1995 (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Sedman, J. G., Smith, J. A., & Struhl, K. eds. (1995). Current Protocols in Molecular Biology. New York: John Wiley and Sons).

Structural similarity between two polynucleotide sequences can be expressed as a function of "stringency" of the conditions under which the two sequences will hybridize with one another. As used herein, the term "stringency" refers to the extent that the conditions disfavor hybridization. Stringent conditions strongly disfavor hybridization, and only the most structurally related molecules will hybridize to one another under such conditions. Conversely, non-stringent conditions favor hybridization of molecules displaying a lesser degree of structural relatedness. Hybridization stringency, therefore, directly correlates with the structural relationships of two nucleic acid sequences. The following relationships are useful in correlating hybridization and relatedness (where  $T_m$  is the melting temperature of a nucleic acid duplex):

- a.  $T_m = 69.3 + 0.41(G+C)\%$
- b. The  $T_m$  of a duplex DNA decreases by  $1^\circ\text{C}$  with every increase of 1% in the number of mismatched base pairs.
- c.  $(T_m)_{\mu 2} - (T_m)_{\mu 1} = 18.5 \log_{10} \mu 2 / \mu 1$   
where  $\mu 1$  and  $\mu 2$  are the ionic strengths of two solutions.

Hybridization stringency is a function of many factors, including overall DNA concentration, ionic strength, temperature, probe size and the presence of agents which disrupt hydrogen bonding. Factors promoting hybridization include

high DNA concentrations, high ionic strengths, low temperatures, longer probe size and the absence of agents that disrupt hydrogen bonding. Hybridization typically is performed in two phases: the "binding" phase and the "washing" phase.

5 First, in the binding phase, the probe is bound to the target under conditions favoring hybridization. Stringency is usually controlled at this stage by altering the temperature. For high stringency, the temperature is usually between 65°C and 70°C, unless short (< 20 nt) oligonucleotide probes are used. A representative hybridization solution comprises 6X SSC, 0.5% SDS, 5X Denhardt's solution and  
10 100 µg of nonspecific carrier DNA. See Ausubel *et al.*, section 2.9, supplement 27 (1994). Of course, many different, yet functionally equivalent, buffer conditions are known. Where the degree of relatedness is lower, a lower temperature may be chosen. Low stringency binding temperatures are between about 25°C and 40°C. Medium stringency is between at least about 40°C to less  
15 than about 65°C. High stringency is at least about 65°C.

Second, the excess probe is removed by washing. It is at this phase that more stringent conditions usually are applied. Hence, it is this "washing" stage that is most important in determining relatedness via hybridization. Washing solutions typically contain lower salt concentrations. One exemplary medium  
20 stringency solution contains 2X SSC and 0.1% SDS. A high stringency wash solution contains the equivalent (in ionic strength) of less than about 0.2X SSC, with a preferred stringent solution containing about 0.1X SSC. The temperatures associated with various stringencies are the same as discussed above for "binding." The washing solution also typically is replaced a number of times  
25 during washing. For example, typical high stringency washing conditions

comprise washing twice for 30 minutes at 55° C. and three times for 15 minutes at 60° C.

The present invention includes nucleic acid molecules that hybridize to the molecules of set forth in Figures 1a and 2a under high stringency binding and washing conditions, where such nucleic molecules encode an antibody or functional fragment thereof having properties as described herein. Preferred molecules (from an mRNA perspective) are those that have at least 75% or 80% (preferably at least 85%, more preferably at least 90% and most preferably at least 95%) homology or sequence identity with one of the DNA molecules described herein. In one particular example of a variant of the invention, nucleic acid position 7 in SEQ ID NOS: 1, 2, 3 and/or 4 can be substituted from a C to an G, thereby changing the codon from CAA to GAA.

#### ***Functionally Equivalent Variants***

Yet another class of DNA variants within the scope of the invention may be described with reference to the product they encode (see the peptides listed in figures 1b and 2b). These functionally equivalent genes characterized by the fact that they encode the same peptide sequences found in figures 1b and 2b due to the degeneracy of the genetic code. SEQ ID NOS: 1 and 31 are an example of functionally equivalent variants, as their nucleic acid sequences are different, yet they encode the same polypeptide, i.e. SEQ ID NO: 5.

It is recognized that variants of DNA molecules provided herein can be constructed in several different ways. For example, they may be constructed as completely synthetic DNAs. Methods of efficiently synthesizing oligonucleotides in the range of 20 to about 150 nucleotides are widely available. See Ausubel *et al.*, section 2.11, Supplement 21 (1993). Overlapping oligonucleotides may be synthesized and assembled in a fashion first reported by Khorana *et al.*, J. Mol.



Biol. 72:209-217 (1971); *see also* Ausubel *et al.*, *supra*, Section 8.2. Synthetic DNAs preferably are designed with convenient restriction sites engineered at the 5' and 3' ends of the gene to facilitate cloning into an appropriate vector.

As indicated, a method of generating variants is to start with one of the  
5 DNAs disclosed herein and then to conduct site-directed mutagenesis. *See* Ausubel *et al.*, *supra*, chapter 8, Supplement 37 (1997). In a typical method, a target DNA is cloned into a single-stranded DNA bacteriophage vehicle. Single-stranded DNA is isolated and hybridized with an oligonucleotide containing the desired nucleotide alteration(s). The complementary strand is synthesized and the  
10 double stranded phage is introduced into a host. Some of the resulting progeny will contain the desired mutant, which can be confirmed using DNA sequencing. In addition, various methods are available that increase the probability that the progeny phage will be the desired mutant. These methods are well known to those in the field and kits are commercially available for generating such mutants.

#### 15 **Recombinant DNA constructs and expression**

The present invention further provides recombinant DNA constructs comprising one or more of the nucleotide sequences of the present invention. The recombinant constructs of the present invention are used in connection with a vector, such as a plasmid or viral vector, into which a DNA molecule encoding an  
20 antibody of the invention is inserted.

The encoded gene may be produced by techniques described in Sambrook *et al.*, 1989, and Ausubel *et al.*, 1989. Alternatively, the DNA sequences may be chemically synthesized using, for example, synthesizers. *See*, for example, the techniques described in OLIGONUCLEOTIDE SYNTHESIS (1984, Gait, ed., IRL  
25 Press, Oxford), which is incorporated by reference herein in its entirety. Recombinant constructs of the invention are comprised with expression vectors

that are capable of expressing the RNA and/or protein products of the encoded DNA(s). The vector may further comprise regulatory sequences, including a promoter operably linked to the open reading frame (ORF). The vector may further comprise a selectable marker sequence. Specific initiation and bacterial  
5 secretory signals also may be required for efficient translation of inserted target gene coding sequences.

The present invention further provides host cells containing at least one of the DNAs of the present invention. The host cell can be virtually any cell for which expression vectors are available. It may be, for example, a higher  
10 eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, but preferably is a prokaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection, electroporation or phage infection.

#### 15 ***Bacterial Expression***

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable  
20 markers and an origin of replication to ensure maintenance of the vector and, if desirable, to provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*.

25 Bacterial vectors may be, for example, bacteriophage-, plasmid- or phagemid-based. These vectors can contain a selectable marker and bacterial

origin of replication derived from commercially available plasmids typically containing elements of the well known cloning vector pBR322 (ATCC 37017). Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is de-repressed/induced by appropriate means (*e.g.*, temperature shift or chemical induction) and cells are  
5 cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

In bacterial systems, a number of expression vectors may be advantageously  
10 selected depending upon the use intended for the protein being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of antibodies or to screen peptide libraries, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable.

15

### **Therapeutic Methods**

Therapeutic methods involve administering to a subject in need of treatment a therapeutically effective amount of an antibody contemplated by the invention. A "therapeutically effective" amount hereby is defined as the amount of an  
20 antibody that is of sufficient quantity to deplete CD38-positive cells in a treated area of a subject—either as a single dose or according to a multiple dose regimen, alone or in combination with other agents, which leads to the alleviation of an adverse condition, yet which amount is toxicologically tolerable. The subject may be a human or non-human animal (*e.g.*, rabbit, rat, mouse, monkey or other lower-  
25 order primate).

An antibody of the invention might be co-administered with known medicaments, and in some instances the antibody might itself be modified. For example, an antibody could be conjugated to an immunotoxin or radioisotope to potentially further increase efficacy.

5       The inventive antibodies can be used as a therapeutic or a diagnostic tool in a variety of situations where CD38 is undesirably expressed or found. Disorders and conditions particularly suitable for treatment with an antibody of the inventions are multiple myeloma (MM) and other haematological diseases, such as chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML),  
10       acute myelogenous leukemia (AML), and acute lymphocytic leukemia (ALL). An antibody of the invention also might be used to treat inflammatory disease such as rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE).

To treat any of the foregoing disorders, pharmaceutical compositions for use in accordance with the present invention may be formulated in a conventional  
15       manner using one or more physiologically acceptable carriers or excipients. An antibody of the invention can be administered by any suitable means, which can vary, depending on the type of disorder being treated. Possible administration routes include parenteral (*e.g.*, intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous), intrapulmonary and intranasal, and, if desired  
20       for local immunosuppressive treatment, intralesional administration. In addition, an antibody of the invention might be administered by pulse infusion, with, *e.g.*, declining doses of the antibody. Preferably, the dosing is given by injections, most preferably intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. The amount to be administered will depend  
25       on a variety of factors such as the clinical symptoms, weight of the individual, whether other drugs are administered. The skilled artisan will recognize that the

route of administration will vary depending on the disorder or condition to be treated.

Determining a therapeutically effective amount of the novel polypeptide, according to this invention, largely will depend on particular patient characteristics, route of administration, and the nature of the disorder being treated. General guidance can be found, for example, in the publications of the International Conference on Harmonisation and in REMINGTON'S PHARMACEUTICAL SCIENCES, chapters 27 and 28, pp. 484-528 (18th ed., Alfonso R. Gennaro, Ed., Easton, Pa.: Mack Pub. Co., 1990). More specifically, determining a therapeutically effective amount will depend on such factors as toxicity and efficacy of the medicament. Toxicity may be determined using methods well known in the art and found in the foregoing references. Efficacy may be determined utilizing the same guidance in conjunction with the methods described below in the Examples.

#### **Diagnostic Methods**

CD38 is highly expressed on hematological cells in certain malignancies; thus, an anti-CD38 antibody of the invention may be employed in order to image or visualize a site of possible accumulation of malignant cells in a patient. In this regard, an antibody can be detectably labeled, through the use of radioisotopes, affinity labels (such as biotin, avidin, etc.) fluorescent labels, paramagnetic atoms, etc. Procedures for accomplishing such labeling are well known to the art. Clinical application of antibodies in diagnostic imaging are reviewed by Grossman, H. B., Urol. Clin. North Amer. 13:465-474 (1986)), Unger, E. C. et al., Invest. Radiol. 20:693-700 (1985)), and Khaw, B. A. et al., Science 209:295-297 (1980)).

The detection of foci of such detectably labeled antibodies might be indicative of a site of tumor development, for example. In one embodiment, this examination is done by removing samples of tissue or blood and incubating such samples in the presence of the detectably labeled antibodies. In a preferred  
5 embodiment, this technique is done in a non-invasive manner through the use of magnetic imaging, fluorography, etc. Such a diagnostic test may be employed in monitoring the success of treatment of diseases, where presence or absence of CD38-positive cells is a relevant indicator.

#### 10 **Therapeutic And Diagnostic Compositions**

The antibodies of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, wherein an antibody of the invention (including any functional fragment thereof) is combined in a mixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles  
15 and their formulation are described, for example, in REMINGTON'S PHARMACEUTICAL SCIENCES (18th ed., Alfonso R. Gennaro, Ed., Easton, Pa.: Mack Pub. Co., 1990). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of one or more of the antibodies of the present invention,  
20 together with a suitable amount of carrier vehicle.

Preparations may be suitably formulated to give controlled-release of the active compound. Controlled-release preparations may be achieved through the use of polymers to complex or absorb anti-CD38 antibody. The controlled delivery may be exercised by selecting appropriate macromolecules (for example  
25 polyesters, polyamino acids, polyvinyl, pyrrolidone, ethylenevinyl-acetate, methylcellulose, carboxymethylcellulose, or protamine, sulfate) and the

concentration of macromolecules as well as the methods of incorporation in order to control release. Another possible method to control the duration of action by controlled release preparations is to incorporate anti-CD38 antibody into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating these agents into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatine-microcapsules and poly(methylmethacrylate) microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (1980).

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules, or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compositions may, if desired, be presented in a pack or dispenser device, which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

The invention further is understood by reference to the following working examples, which are intended to illustrate and, hence, not limit the invention.



## EXAMPLES

### Cell-lines

The following cell-lines were obtained from the European Collection of Cell Cultures (ECACC), the German Collection of Microorganisms (DSMZ) or the  
5 American Type Culture collection (ATCC): hybridoma cell line producing the CD38 mouse IgG1 monoclonal antibody OKT10 (ECACC, #87021903), Jurkat cells (DSMZ, ACC282), LP-1 (DSMZ, ACC41), RPMI8226 (ATCC, CCL-155), HEK293 (ATCC, CRL-1573), CHO-K1 (ATCC, CRL-61) and Raji (ATCC, CCL-86)

10

### Cells and culture-conditions

All cells were cultured under standardized conditions at 37°C and 5% CO<sub>2</sub> in a humidified incubator. The cell-lines LP-1, RPMI8226, Jurkat and Raji were cultured in RPMI1640 (Pan biotech GmbH, #P04-16500) supplemented with  
15 10 % FCS (PAN biotech GmbH, #P30-3302), 50 U/ml penicillin, 50 µg/ml streptomycin (Gibco, #15140-122) and 2 mM glutamine (Gibco, #25030-024) and, in case of Jurkat- and Raji-cells, additionally 10 mM Hepes (Pan biotech GmbH, #P05-01100) and 1 mM sodium pyruvate (Pan biotech GmbH, # P04-43100) had to be added.

20 CHO-K1 and HEK293 were grown in DMEM (Gibco, #10938-025) supplemented with 2 mM glutamine and 10% FCS. Stable CD38 CHO-K1 transfectants were maintained in the presence of G418 (PAA GmbH, P11-012) whereas for HEK293 the addition of 1mM sodium-pyruvate was essential. After transient transfection of HEK293 the 10% FCS was replaced by Ultra low IgG FCS (Invitrogen,  
25 #16250-078). The cell-line OKT10 was cultured in IDMEM (Gibco, #31980-022), supplemented with 2 mM glutamine and 20 % FCS.

### Preparation of single cell suspensions from peripheral blood

All blood samples were taken after informed consent. Peripheral blood  
30 mononuclear cells (PBMC) were isolated by Histopaque®-1077 (Sigma) according to the manufacturer's instructions from healthy donors. Red blood cells were depleted from these cell suspensions by incubation in ACK Lysis Buffer (0.15 M NH<sub>4</sub>Cl, 10 mM KHCO<sub>3</sub>, 0.1 M EDTA) for 5 min at RT or a commercial

derivative (Bioscience, #00-4333). Cells were washed twice with PBS and then further processed for flow cytometry or ADCC (see below).

#### Flow cytometry ("FACS")

- 5 All stainings were performed in round bottom 96-well culture plates (Nalge Nunc) with  $2 \times 10^5$  cells per well. Cells were incubated with Fab or IgG antibodies at the indicated concentrations in 50  $\mu$ l FACS buffer (PBS, 3% FCS, 0.02% NaN<sub>3</sub>) for 40 min at 4°C. Cells were washed twice and then incubated with R-Phycoerythrin (PE) conjugated goat-anti-human or goat-anti-mouse IgG (H+L) F(ab')<sub>2</sub> (Jackson
- 10 Immuno Research), diluted 1:200 in FACS buffer, for 30 min at 4°C. Cells were again washed, resuspended in 0.3 ml FACS buffer and then analyzed by flow cytometry in a FACSCalibur (Becton Dickinson, San Diego, CA).
- For FACS based Scatchard analyses RPMI8226 cells were stained with at 12 different dilutions (1:2<sup>n</sup>) starting at 12.5  $\mu$ g/ml (IgG) final concentration. At least
- 15 two independent measurements were used for each concentration and K<sub>D</sub> values extrapolated from median fluorescence intensities according to Chamow et al. (1994).

#### Surface plasmon resonance

- 20 The kinetic constants k<sub>on</sub> and k<sub>off</sub> were determined with serial dilutions of the respective Fab binding to covalently immobilized CD38-Fc fusion protein using the BIAcore 3000 instrument (Biacore, Uppsala, Sweden). For covalent antigen immobilization standard EDC-NHS amine coupling chemistry was used. For direct coupling of CD38 Fc-fusion protein CM5 sensor chips (Biacore) were
- 25 coated with ~600-700 RU in 10 mM acetate buffer, pH 4.5. For the reference flow cell a respective amount of HSA (human serum albumin) was used. Kinetic measurements were done in PBS (136 mM NaCl, 2.7 mM KCl, 10mM Na<sub>2</sub>HPO<sub>4</sub>, 1.76 mM KH<sub>2</sub>PO<sub>4</sub> pH 7.4) at a flow rate of 20  $\mu$ l/min using Fab concentration range from 1.5-500 nM. Injection time for each concentration was 1 min,
- 30 followed by 2 min dissociation phase. For regeneration 5  $\mu$ l 10mM HCl was used. All sensograms were fitted locally using BIA evaluation software 3.1 (Biacore).

**EXAMPLE 1: Antibody Generation from HuCAL Libraries**

For the generation of therapeutic antibodies against CD38, selections with the MorphoSys HuCAL GOLD phage display library were carried out. HuCAL GOLD<sup>®</sup> is a Fab library based on the HuCAL<sup>®</sup> concept (Knappik et al., 2000; Krebs et al., 2001), in which all six CDRs are diversified, and which employs the CysDisplay<sup>™</sup> technology for linking Fab fragments to the phage surface (Löhning, 2001).

**A. Phagemid rescue, phage amplification and purification**

HuCAL GOLD<sup>®</sup> phagemid library was amplified in 2 x TY medium containing 34 µg/ml chloramphenicol and 1 % glucose (2 x TY-CG). After helper phage infection (VCSM13) at an OD600 of 0.5 (30 min at 37°C without shaking; 30 min at 37°C shaking at 250 rpm), cells were spun down (4120 g; 5 min; 4°C), resuspended in 2 x TY / 34 µg/ml chloramphenicol / 50 µg/ml kanamycin and grown overnight at 22°C. Phages were PEG-precipitated from the supernatant, resuspended in PBS / 20 % glycerol and stored at -80°C. Phage amplification between two panning rounds was conducted as follows: mid-log phase TG1 cells were infected with eluted phages and plated onto LB-agar supplemented with 1 % of glucose and 34 µg/ml of chloramphenicol (LB-CG). After overnight incubation at 30°C, colonies were scraped off, adjusted to an OD600 of 0.5 and helper phage added as described above.

**B. Pannings with HuCAL GOLD<sup>®</sup>**

For the selections HuCAL GOLD<sup>®</sup> antibody-phages were divided into three pools corresponding to different VH master genes (pool 1: VH1/5λκ, pool 2: VH3 λκ, pool 3: VH2/4/6 λκ). These pools were individually subjected to 3 rounds of whole cell panning on CD38-expressing CHO-K1 cells followed by pH-elution and a post-adsorption step on CD38-negative CHO-K1-cells for depletion of irrelevant antibody-phages. Finally, the remaining antibody phages were used to

infect E. coli TG1 cells. After centrifugation the bacterial pellet was resuspended in 2 x TY medium, plated on agar plates and incubated overnight at 30°C. The selected clones were then scraped from the plates, phages were rescued and amplified. The second and the third round of selections was performed as the  
5 initial one.

The Fab encoding inserts of the selected HuCAL GOLD<sup>®</sup> phages were subcloned into the expression vector pMORPH<sup>®</sup>x9\_Fab\_FS (Rauchenberger et al., 2003) to facilitate rapid expression of soluble Fab. The DNA of the selected clones was digested with XbaI and EcoRI thereby cutting out the Fab encoding insert (ompA-  
10 VLCL and phoA-Fd), and cloned into the XbaI / EcoRI cut vector pMORPH<sup>®</sup>x9\_Fab\_FS. Fab expressed in this vector carry two C-terminal tags (FLAG<sup>™</sup> and Strep-tag<sup>®</sup> II) for detection and purification.

#### **EXAMPLE 2: Biological assays**

15 Antibody dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity was measured according to a published protocol based on flow-cytometry analysis (Naundorf et al., 2002) as follows:

##### **ADCC:**

20 For ADCC measurements, target cells (T) were adjusted to 2.0E+05 cells/ml and labeled with 100 ng/ml Calcein AM (Molecular Probes, C-3099) in RPMI1640 medium (Pan biotech GmbH) for 2 minutes at room temperature. Residual calcein was removed by 3 washing steps in RPMI1640 medium. In parallel PBMC were prepared as source for (natural killer) effector cells (E), adjusted to 1.0E+07 and  
25 mixed with the labeled target cells to yield a final E:T-ratio of 50:1 or less, depending on the assay conditions. Cells were washed once and the cell-mix

resuspended in 200 µl RPMI1640 medium containing the respective antibody at different dilutions. The plate was incubated for 4 hrs under standardized conditions at 37°C and 5% CO<sub>2</sub> in a humidified incubator. Prior to FACS analysis cells were labeled with propidium-iodide (PI) and analyzed by flow-cytometry  
5 (Becton-Dickinson). Between 50.000 and 150.000 events were counted for each assay.

The following equation gave rise to the killing activity [in %]:

10

$$\frac{ED^A}{EL^A + ED^A} \times 100$$

with ED<sup>A</sup> = events dead cells (calcein + PI stained cells), and  
EL<sup>A</sup> = events living cells (calcein stained cells)

15

CDC:

For CDC measurements, 5.0E+04 CD38 CHO-K1 transfectants were added to a microtiter well plate (Nunc) together with a 1:4 dilution of human serum (Sigma, #S-1764) and the respective antibody. All reagents and cells were diluted in  
20 RPMI1640 medium (Pan biotech GmbH) supplemented with 10% FCS. The reaction-mix was incubated for 2 hrs under standardized conditions at 37°C and 5% CO<sub>2</sub> in a humidified incubator. As negative controls served either heat-inactivated complement or CD38-transfectants without antibody. Cells were labeled with PI and subjected to FACS-analysis.

25 In total 5000 events were counted and the number of dead cells at different antibody concentrations used for the determination of EC50 values. The following equation gave rise to the killing activity [in %]:

30

$$\frac{ED^C}{EL^C + ED^C} \times 100$$

with ED<sup>C</sup> = events dead cells (PI stained cells), and  
EL<sup>C</sup> = events living cells (unstained)

Cytotoxicity values from a total of 12 different antibody-dilutions ( $1:2^n$ ) in triplicates were used in ADCC and duplicates in CDC for each antibody in order obtain EC-50 values with a standard analysis software (PRISM<sup>®</sup>, Graph Pad Software).

**EXAMPLE 3: Generation of stable CD38-transfectants and CD38 Fc-fusion proteins**

In order to generate CD38 protein for panning and screening two different expression systems had to be established. The first strategy included the generation of CD38-Fc-fusion protein which was purified from supernatants after transient transfection of HEK293 cells. The second strategy involved the generation of a stable CHO-K1 -cell line for high CD38 surface expression to be used for selection of antibody-phages via whole cell panning.

As an initial step Jurkat cells (DSMZ ACC282) were used for the generation of cDNA (Invitrogen) followed by amplification of the entire CD38-coding sequence using primers complementary to the first 7 and the last 9 codons of CD38, respectively (primer MTE001 & MTE002rev; Table 4). Sequence analysis of the CD38-insert confirmed the published amino acid sequence by Jackson et al. (1990) except for position 49 which revealed a glutamine instead of a tyrosine as described by Nata et al. (1997). For introduction of restriction endonuclease sites and cloning into different derivatives of expression vector pcDNA3.1 (Stratagene), the purified PCR-product served as a template for the re-amplification of the entire gene (primers MTE006 & MTE007rev, Table 4) or a part (primers MTE004 & MTE009rev, Table 4) of it. In the latter case a fragment encoding for the extracellular domain (aa 45 to 300) was amplified and cloned in frame between a human V $\kappa$  leader sequence and a human Fc-gamma 1 sequence. This vector served as expression vector for the generation of soluble CD38-Fc fusion-protein. Another pcDNA3.1-derivative without leader-sequence was used for insertion of the CD38 full-length gene. In this case a stop codon in front of the Fc-coding region and the missing leader-sequence gave rise to CD38-surface expression. HEK293 cells were transiently transfected with the Fc-fusion protein vector for generation of soluble CD38 Fc-fusion protein and, in case of the full-length derivative, CHO-K1-cells were transfected for the generation of a stable CD38-expressing cell line.

**Table 4:**

Primer #	Sequence (5'-> 3')
MTE001	ATG GCC AAC TGC GAG TTC AGC (SEQ ID NO: 25)
MTE002rev	TCA GAT CTC AGA TGT GCA AGA TGA ATC (SEQ ID NO: 26)
MTE004	TT GGT ACC AGG TGG CGC CAG CAG TG (SEQ ID NO: 27)
MTE006	TT GGT ACC ATG GCC AAC TGC GAG (SEQ ID NO: 28)
MTE007rev	CCG ATA TCA* GAT CTC AGA TGT GCA AGA TG (SEQ ID NO: 29)
MTE009rev	CCG ATA TC GAT CTC AGA TGT GCA AGA TG (SEQ ID NO: 30)

\* leading to a stop codon (TGA) in the sense orientation.

#### EXAMPLE 4: Cloning, expression and purification of HuCAL<sup>®</sup> IgG1:

- 5 In order to express full length IgG, variable domain fragments of heavy (VH) and light chains (VL) were subcloned from Fab expression vectors into appropriate pMORPH<sup>®</sup>\_hlg vectors (see Figures 8 to 10). Restriction endonuclease pairs *BlnI/MfeI* (insert-preparation) and *BlnI/EcoRI* (vector-preparation) were used for subcloning of the VH domain fragment into pMORPH<sup>®</sup>\_hIgG1. Enzyme-pairs
- 10 *EcoRV/HpaI* (lambda-insert) and *EcoRV/BsiWI* (kappa-insert) were used for subcloning of the VL domain fragment into the respective pMORPH<sup>®</sup>\_hIgκ\_1 or pMORPH<sup>®</sup>\_h\_Igλ\_1 vectors. Resulting IgG constructs were expressed in HEK293 cells (ATCC CRL-1573) by transient transfection using standard calcium phosphate –DNA coprecipitation technique.
- 15 IgGs were purified from cell culture supernatants by affinity chromatography *via* Protein A Sepharose column. Further down stream processing included a buffer exchange by gel filtration and sterile filtration of purified IgG. Quality control revealed a purity of >90 % by reducing SDS-PAGE and >90 % monomeric IgG as determined by analytical size exclusion chromatography. The endotoxin content
- 20 of the material was determined by a kinetic LAL based assay (Cambrex European Endotoxin Testing Service, Belgium).



**EXAMPLE 5: Generation and production of chimeric OKT10 (chOKT10; SEQ ID NOS: 23 and 24)**

For the construction of chOKT10 the mouse VH and VL regions were amplified by PCR using cDNA prepared from the murine OKT10 hybridoma cell line (ECACC #87021903). A set of primers were used as published (Dattamajumdar et al., 1996; Zhou et al., 1994). PCR products were used for Topo-cloning (Invitrogen; pCRII-vector) and single colonies subjected to sequence analysis (M13 reverse primer) which revealed two different kappa light chain sequences and one heavy chain sequence. According to sequence alignments (EMBL-nucleotide sequence database) and literature (Krebber et al, 1997) one of the kappa-sequence belongs to the intrinsic repertoire of the tumor cell fusion partner X63Ag8.653 and hence does not belong to OKT10 antibody. Therefore, only the new kappa sequence and the single VH-fragment was used for further cloning. Both fragments were reamplified for the addition of restriction endonuclease sites followed by cloning into the respective pMORPH<sup>®</sup> IgG1-expression vectors. The sequences for the heavy chain (SEQ ID NO: 23) and light chain (SEQ ID NO: 24) are given in Fig. 6. HEK293 cells were transfected transiently and the supernatant analyzed in FACS for the chimeric OKT10 antibody binding to the CD38 over-expressing Raji cell line (ATCC).

**EXAMPLE 6: Epitope Mapping****1. Materials and Methods:****Antibodies:**

The following anti-CD38 IgGs were sent for epitope mappings:

MOR#	Lot #	Format	Conc. [mg/ml]/Vol.[μl]
MOR03077	2CHE106_030602	human IgG1	0.44/1500
MOR03079	2APO31	human IgG1	0.38/500
MOR03080	030116_4CUE16	human IgG1	2.28/200
MOR03100	030612_6SBA6	human IgG1	0.39/500
chim. OKT10*	030603_2CHE111	human IgG1	0.83/500

\* chimeric OKT10 consisting of human Fc and mouse variable regions.

**CD38-Sequence:**

The amino acid (aa) sequence (position 44 – 300) is based on human CD38 taken from the published sequence under SWISS-PROT primary accession number P28907. At position 49 the aa Q (instead of T) has been used for the peptide-design.

**PepSpot-Analysis:**

The antigen peptides were synthesized on a cellulose membrane in a stepwise manner resulting in a defined arrangement (peptide array) and are covalently bound to the cellulose membrane. Binding assays were performed directly on the peptide array.

In general an antigen peptide array is incubated with blocking buffer for several hours to reduce non-specific binding of the antibodies. The incubation with the primary (antigen peptide-binding) antibody in blocking buffer occurs followed by the incubation with the peroxidase (POD)-labelled secondary antibody, which binds selectively the primary antibody. A short T(Tween)-TBS-buffer washing directly after the incubation of the antigen peptide array with the secondary antibody followed by the first chemiluminescence experiment is made to get a first overview which antigen peptides do bind the primary antibody. Several buffer washing steps follow (T-TBS- and TBS-buffer) to reduce false positive binding (unspecific antibody binding to the cellulose membrane itself). After these washing steps the final chemiluminescence analysis is performed. The data were analysed with an imaging system showing the signal intensity (Boehringer Light units, BLU) as single measurements for each peptide. In order to evaluate non-specific binding of the secondary antibodies (anti-human IgG), these antibodies were incubated with the peptide array in the absence of primary antibodies as the first step. If the primary antibody does not show any binding to the peptides it can be directly labelled with POD, which increases the sensitivity of the system (as performed for MOR3077). In this case a conventional coupling chemistry *via* free amino-groups is performed.

The antigen was scanned with 13-mer peptides (11 amino acids overlap). This resulted in arrays of 123 peptides. Binding assays were performed directly on the array. The peptide-bound antibodies MOR03077, MOR03079, MOR03080, MOR03100 and chimeric OKT10 were detected using a peroxidase-labelled

secondary antibody (peroxidase conjugate-goat anti-human IgG, gamma chain specific, affinity isolated antibody; Sigma-Aldrich, A6029). The mappings were performed with a chemiluminescence substrate in combination with an imaging system. Additionally, a direct POD-labelling of MOR03077 was performed in  
5 order to increase the sensitivity of the system.

## 2. Summary and Conclusions:

All five antibodies showed different profiles in the PepSpot analysis. A schematic summary is given in Fig. 7, which illustrates the different aa sequences of CD38  
10 being recognized. The epitope for MOR03079 and chimeric OKT10 can clearly be considered as linear. The epitope for MOR03079 can be postulated within aa 192 – 206 (VSRRFAEAACDVVHV) of CD38 whereas for chimeric OKT10 a sequence between aa 284 and 298 (FLQCVKNPEDSSCTS) is recognized predominantly. The latter results confirm the published data for the parental  
15 murine OKT10 (Hoshino *et al.*, 1997), which postulate its epitope between aa 280-298. Yet, for a more precise epitope definition and determination of key amino acids (main antigen-antibody interaction sites) a shortening of peptides VSRRFAEAACDVVHV and FLQCVKNPEDSSCTS and an alanine-scan of both should be envisaged.

20 The epitopes for MOR03080 and MOR03100 can be clearly considered as discontinuous since several peptides covering different sites of the protein sites were recognized. Those peptides comprise aa 82-94 and aa 158-170 for MOR03080 and aa 82-94, 142-154, 158-170, 188-200 and 280-296 for MOR03100. However, some overlaps between both epitopes can be postulated  
25 since two different sites residing within aa positions 82-94 (CQSVWDAFKGAFI; peptide #20) and 158-170 (TWCGEFNTSKINY; peptide #58) are recognized by both antibodies.

The epitope for MOR03077 can be considered as clearly different from the latter two and can be described as multisegmented discontinuous epitope. The  
30 epitope includes aa 44-66, 110-122, 148-164, 186-200 and 202-224.

**EXAMPLE 7: IL-6-release/proliferation assay****1. Materials and Methods:**

Proliferation- and a IL-6 release assays have been performed according to Ausiello et al. (2000) with the following modifications: PBMCs from different  
5 healthy donors (after obtaining informed consent) were purified by density gradient centrifugation using the Histopaque cell separation system according to the instructions of the supplier (Sigma) and cultured under standard conditions (5% CO<sub>2</sub>, 37°C) in RPMI1640 medium, supplemented with 10% FCS and glutamine ("complete RPMI1640"). For both assays the following antibodies were  
10 used: HuCAL® anti-CD38 IgG1s Mabs MOR03077, MOR03079, and MOR03080, an agonistic murine IgG2a monoclonal antibody (IB4; Malavasi et al., 1984), an irrelevant HuCAL® IgG1 antibody, a matched isotype control (murine IgG2a: anti-trinitrophenol, hapten-specific antibody; cat.#: 555571, clone G155-178; Becton Dickinson) or a medium control. For the IL-6 release assay,  
15 1.0 E+06 PBMCs in 0.5 ml complete RPMI1640 medium were incubated for 24 hrs in a 15 ml culture tube (Falcon) in the presence of 20 µg/ml antibodies. Cell culture supernatants were harvested and analysed for IL-6 release using the Quantikine kit according to the manufacturer's protocol (R&D systems). For the proliferation assay 2.0E+05 PBMCs were incubated for 3 days in a 96-well flat  
20 bottom plate (Nunc) in the presence of 20 µg/ml antibodies. Each assay was carried out in duplicates. After 4 days BrdU was added to each well and cells incubated for an additional 24 hrs at 37°C prior to cell fixation and DNA denaturation according to the protocol of the supplier (Roche). Incorporation of BrdU was measured via an anti-BrdU peroxidase-coupled antibody in a  
25 chemiluminescence-based setting.

**2. Summary and Conclusions:****Proliferation Assay:**

30 In addition to its catalytic activities as a cyclic ADP-ribose cyclase and hydrolase, CD38 displays the ability to transduce signals of biological relevance (Hoshino et al., 1997; Ausiello et al., 2000). Those functions can be induced in vivo by e.g. receptor-ligand interactions or by cross-linking with anti-CD38 antibodies. Those signalling events lead e.g. to calcium mobilization, lymphocyte proliferation and

release of cytokines. However, this signalling is not only dependent on the antigenic epitope but might also vary from donor to donor (Ausiello et al., 2000).

In the view of immunotherapy non-agonistic antibodies are preferable over agonistic antibodies. Therefore, HuCAL® anti-CD38 antibodies (Mabs

5 MOR03077; MOR03079, MOR03080) were further characterized in a proliferation assay and IL-6- (important MM growth-factor) release assay in comparison to the reference antibody chOKT10 and the agonistic anti-CD38 monoclonal antibody IB4.

As demonstrated in Fig.11 and Fig. 12 the HuCAL anti-CD38 antibodies Mab#1,  
10 2 and 3 as well as the reference antibody chOKT10 and corresponding negative controls showed no or only weak induction of proliferation and no IL-6-release as compared to the agonistic antibody IB4.

#### **EXAMPLE 8: Clonogenic assay**

##### **15 1. Materials and Methods:**

PBMCs harbouring autologous CD34+/CD38+ precursor cells were isolated from healthy individuals (after obtaining informed consent) by density gradient centrifugation using the Histopaque cell separation system according to the instructions of the supplier (Sigma) and incubated with different HuCAL®  
20 IgG1 anti-CD38 antibodies (Mabs MOR03077, MOR03079, and MOR03080) and the positive control (PC) chOKT10 at 10 µg/ml. Medium and an irrelevant HuCAL® IgG1 served as background control. Each ADCC-assay consisted of 4.0E+05 PBMCs which were incubated for 4 hrs at 37°C in RPMI1640 medium supplemented with 10% FCS. For the clonogenic assay 2.50 ml “complete”  
25 methylcellulose (CellSystems) was inoculated with 2.5 E+05 cells from the ADCC-assay and incubated for colony-development for at least 14 days in a controlled environment (37°C; 5% CO<sub>2</sub>). Colonies were analyzed by two independent operators and grouped into BFU-E + CFU-GEMM (erythroid burst forming units and granulocyte/ erythroid / macrophage / megakaryocyte stem  
30 cells) and CFU-GM (granulocyte / macrophage stem cells).

##### **2. Summary and Conclusions:**

Since CD38-expression is not only found on immune cells within the myeloid (e.g. monocytes, granulocytes) and lymphoid lineage (e.g. activated B and T-cells;

plasma cells) but also on the respective precursor cells (CD34+/CD38+), it is important that those cells are not affected by antibody-mediated killing.

Therefore, a clonogenic assay was applied in order to analyse those effects on CD34+/CD38+ progenitors.

- 5 PBMCs from healthy donors were incubated with HuCAL® anti-CD38 antibodies (Mab#1, Mab#2 and Mab#3) or several controls (irrelevant HuCAL® antibody, medium and reference antibody chOKT10 as positive control) according to a standard ADCC-protocol followed by further incubation in conditioned methylcellulose for colony-development. As shown in Fig. 13 no significant  
10 reduction of colony-forming units are shown for all HuCAL® anti-CD38 antibodies as compared to an irrelevant antibody or the reference antibody.

#### **EXAMPLE 9: ADCC Assays with different cell-lines and primary multiple myeloma cells**

15 **1. Materials and Methods:**

Isolation and ADCC of MM-patient samples: Bone marrow aspirates were obtained from multiple myeloma patients (after obtaining informed consent). Malignant cells were purified via a standard protocol using anti-CD138 magnetic beads (Miltenty Biotec) after density gradient centrifugation (Sigma). An ADCC-  
20 assay was performed as described before.

**2. Summary and Conclusions:**

Several cell-lines derived from different malignancies were used in ADCC in order to show the cytotoxic effect of the HuCAL® anti-CD38 antibodies on a  
25 broader spectrum of cell-lines including different origins and CD38 expression-levels. As shown in Figure 14, all cells were killed in ADCC at constant antibody concentrations (5 µg/ml) and E:T ratios at 30:1. Cytotoxicity via ADCC was also shown for several multiple myeloma samples from patients. All HuCAL® anti-CD38 antibodies were able to perform a dose-dependent killing of MM-cells and  
30 the EC50-values varied between 0.006 and 0.249 nM (Figure 15).

**EXAMPLE 10: Cross-reactivity analysis by FACS and immunohistochemistry (IHC)****1. Materials and Methods:**

IHC with tonsils: For IHC HuCAL® anti-CD38 Mabs and an irrelevant  
5 negative control antibody were converted into the bivalent dHLX-format  
(Plückthun & Pack, 1997). 5 µm cryo sections from lymph nodes derived from  
Cynomolgus monkey, Rhesus monkey and humans (retrieved from the archives of  
the Institute of Pathology of the University of Graz/Austria) were cut with a Leica  
CM3050 cryostat. Sections were air-dried for 30 minutes to 1 hour and fixed in  
10 ice-cold methanol for 10 minutes and washed with PBS. For the detection of the  
dHLX-format a mouse anti-His antibody (Dianova) in combination with the  
Envision Kit (DAKO) was used. For the detection of the anti-CD38 mouse  
antibodies (e.g. reference mouse monoclonal OKT10) the Envision kit was used  
only.

15 FACS-analysis of lymphocytes: EDTA-treated blood samples were obtained  
from healthy humans (after obtaining informed consent), from Rhesus and  
Cynomolgus monkeys and subjected to density gradient centrifugation using the  
Histopaque cell separation system according to the instructions of the supplier  
(Sigma). For FACS-analysis cells from the interphase were incubated with  
20 primary antibodies (HuCAL® anti-CD38 and negative control Mabs as murine  
IgG2a or Fab-format, the positive control murine antibody OKT10 and a matched  
isotype control) followed by incubation with anti-M2 Flag (Sigma; only for Fab-  
format) and a phycoerythrin (PE)-labeled anti-mouse conjugate (Jackson  
Research). FACS analysis was performed on the gated lymphocyte population.

25

**2. Summary and Conclusions:**

HuCAL® anti-CD38 were analyzed for inter-species CD38 cross-reactivity.  
Whereas all anti-CD38 Mabs were able to detect human CD38 on lymphocytes in  
FACS and IHC, only MOR03080 together with the positive control OKT10  
30 showed an additional reactivity with Cynomolgus and Rhesus monkey CD38 (see  
Table 5: Cross-reactivity analysis).

**Table 5:**

<b>Antibody</b>	<b>Lymphocytes (FACS) and lymph-nodes (IHC) from:</b>		
	<b>Human</b>	<b>Cynomolgus Monkey</b>	<b>Rhesus Monkey</b>
<b>Mab#1</b>	++	-	-
<b>Mab#2</b>	++	-	-
<b>Mab#3</b>	++	++	++
<b>PC</b>	++	++	++
<b>NC</b>	-	-	-

++: strong positive staining; -: no staining; NC: negative control; PC: positive control (=reference cMAb)

## 5 References:

- Ausiello C.M., Urbani F., Lande R., la Sala A., Di Carlo B., Baj G., Surico N., Hilgers J., Deaglio S., Funaro A., Malavasi F. (2000) Functional topography of discrete domains of human CD38. *Tissue Antigens*. 2000 Dec;56(6):539-47.
- 10 Chamow, S.M., Zhang, D.Z., Tan, X.Y, Mathre, S.M., Marsters, S.A., Peers, D.H., Byrn, R.A., Ashknazi, A., Junghans, R.P (1994). humanized, bispecific immunoadhesin-antibody that retargets CD3+ effectors to kill HIV-1-infected cells. *J Immunol*. 1994 Nov 1;153(9):4268-80
- 15 Dattamajumdar, A.K., Jacobsen, D.P., Hood, L.E., Osman, G.E. (1996). Rapid cloning of rearranged mouse immunoglobulin variable genes. *Immunogenetics* 43, 141-151
- 20 Funaro, A., Spagnoli, G.C., Ausiello, C.M., Alessio, M., Roggero, S., Delia, D., Zaccolo, M., and Malavasi, F. (1990) Involvement of the multilineage CD38 molecule in a unique pathway of cell activation and proliferation. *J. Immunol*. 145, 2390-2396.
- 25 Hoshino S., Kukimoto I., Kontani K., Inoue S., Kanda Y., Malavasi F., Katada T. (1997) Mapping of the catalytic and epitopic sites of human CD38/NAD+ glycohydrolase to a functional domain in the carboxyl terminus. *J Immunol*. 158(2):741-7.
- 30 Jackson D.G., Bell J.I. (1990) Isolation of a cDNA encoding the human CD38 (T10) molecule, a cell surface glycoprotein with an unusual discontinuous pattern of expression during lymphocyte differentiation. *J Immunol*. 144(7):2811-5.



- Knappik, A., Ge, L., Honegger, A., Pack, P., Fischer, M., Wellnhofer, G., Hoess, A., Wolle, J., Plückthun, A., and Virnekas, B. (2000). Fully synthetic human combinatorial antibody libraries (HuCAL) based on modular consensus frameworks and CDRs randomized with trinucleotides. *J Mol Biol* 296, 57-86.
- 5 Konopleva M., Estrov Z., Zhao S., Andreeff M., Mehta K. (1998) Ligation of cell surface CD38 protein with agonistic monoclonal antibody induces a cell growth signal in myeloid leukemia cells. *J Immunol.* 161(9):4702-8.
- 10 Krebber, A., Bornhauser, S., Burmester, J., Honegger, A., Willuda, J., Bossard, H.R., Plückthun, A. (1997). Reliable cloning of functional antibody variable domains from hybridomas and spleen cell repertoires employing a reengineered phage display system. *J. Imm. Meth.* 201, 35-55.
- 15 Krebs, B., Rauchenberger, R., Reiffert, S., Rothe, C., Tesar, M., Thomassen, E., Cao, M., Dreier, T., Fischer, D., Hoss, A., Inge, L., Knappik, A., Marget, M., Pack, P., Meng, X.Q., Schier, R., Sohlmann, P., Winter, J., Wolle, J., and Kretzschmar, T. (2001). High-throughput generation and engineering of recombinant human antibodies. *J Immunol Methods* 254, 67-84.
- 20 Löhning, C. (2001). Novel methods for displaying (poly)peptides/proteins on bacteriophage particles via disulfide bonds. WO 01/05950.
- 25 Malavasi, F., Caligaris-Cappio, F., Milanese, C., Dellabona, P., Richiardi, P., Carbonara, A. O. (1984). Characterization of a murine monoclonal antibody specific for human early lymphohemopoietic cells. *Hum. Immunol.* 9: 9-20
- 30 Namba, M., Otsuki, T., Mori, M., Togawa, A., Wada, H., Sugihara, T., Yawata, Y., Kimoto, T. (1989). Establishment of five human myeloma cell lines. *In Vitro Cell Dev. Biol.* 25: 723.
- 35 Nata K., Takamura T., Karasawa T., Kumagai T., Hashioka W., Tohgo A., Yonekura H., Takasawa S., Nakamura S., Okamoto H. (1997). Human gene encoding CD38 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase): organization, nucleotide sequence and alternative splicing. *Gene* 186(2):285-92.
- 40 Naundorf, S., Preithner, S., Mayer, P., Lippold, S., Wolf, A., Hanakam, F., Fichtner, I., Kufer, P., Raum, T., Riethmüller, G., Baeuerle, P.A., Dreier, T. (2002). *Int. J. Cancer* 100, 101-110.
- Plückthun A, and Pack P. (1997) New protein engineering approaches to multivalent and bispecific antibody fragments. *Immunotechnology* 3(2):83-105.
- 45 Rauchenberger R., Borges E., Thomassen-Wolf E., Rom E., Adar R., Yaniv Y., Malka M., Chumakov I., Kotzer S., Resnitzky D., Knappik A., Reiffert S., Prassler J., Jury K., Waldherr D., Bauer S., Kretzschmar T., Yayon A., Rothe C. (2003). Human combinatorial Fab library yielding specific and functional antibodies against the human fibroblast growth factor receptor 3. *J Biol Chem.* 278(40):38194-205.
- 50

Zhou, H., Fisher, R.J., Papas, T.S. (1994). Optimization of primer sequences for mouse scFv repertoire display library construction. *Nucleic Acids Res.* 22: 888-889.

5

10

15

**CLAIMS**

1. An isolated human antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of CD38 (SEQ ID NO: 22), wherein said human antibody or functional fragment thereof is able to mediate killing of a CD38+ target cell by ADCC with an at least five-fold better efficacy than chimeric OKT10 (SEQ ID NOS: 23 and 24) under the same or substantially the same conditions when a human PBMC cell is employed as an effector cell, wherein said CD38+ target cell is selected from the group consisting of LP-1 (DSMZ: ACC41) and RPMI-8226 (ATCC: CCL-155), and wherein the ratio of effector cells to target cells is between about 30:1 and about 50:1.

2. An antigen-binding region of a human antibody or functional fragment thereof according to claim 1.

3. An antigen-binding region according to claim 2, which comprises an H-CDR3 region depicted in SEQ ID NO: 5, 6, 7, or 8.

4. An antigen-binding region according to claim 3, further comprising an H-CDR2 region depicted in SEQ ID NO: 5, 6, 7, or 8.

5. An antigen-binding region according to claim 4, further comprising an H-CDR1 region depicted in SEQ ID NO: 5, 6, 7, or 8.

6. An antigen-binding region according to claim 5, which comprises a variable heavy chain depicted in SEQ ID NO: 5, 6, 7, or 8.

7. An antigen-binding region according to claim 2, which comprises an L-CDR3 region depicted in SEQ ID NO: 13, 14, 15, or 16.

8. An antigen-binding region according to claim 7, further comprising an L-CDR1 region depicted in SEQ ID NO: 13, 14, 15, or 16.

9. An antigen-binding region according to claim 8, further comprising an L-CDR2 region depicted in SEQ ID NO: 13, 14, 15, or 16.

10. An antigen-binding region according to claim 9, which comprises a variable light chain depicted in SEQ ID NO: 13, 14, 15, or 16.

11. An antigen-binding region according to claim 2, which comprises a heavy chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 5, 6, 7, or 8; (ii) a sequence having at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 6, 7, or 8; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 6, 7, or 8.

12. An antigen-binding region according to claim 2, which comprises a light chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 13, 14, 15, or 16; (ii) a sequence having at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 14, 15, or 16; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 14, 15, or 16.

13. An antibody to according to claims 1, which is an IgG.

14. An antibody to according to claim 13, which is an IgG1.

15. An isolated human antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of CD38 (SEQ ID NO: 22), wherein said human antibody or functional fragment thereof is able to mediate killing of a CD38-transfected CHO cell by CDC with an at least two-fold better efficacy than chimeric OKT10 (SEQ ID NOS: 23 and 24) under the same or substantially the same conditions.

16. An antigen-binding region of a human antibody or functional fragment thereof according to claim 15.

17. An antigen-binding region according to claim 16, which comprises an H-CDR3 region depicted in SEQ ID NO: 5, 6, or 7.

18. An antigen-binding region according to claim 17, further comprising an H-CDR2 region depicted in SEQ ID NO: 5, 6, or 7.

19. An antigen-binding region according to claim 18, further comprising an H-CDR1 region depicted in SEQ ID NO: 5, 6, or 7.

20. An antigen-binding region according to claim 19, which comprises a variable heavy chain depicted in SEQ ID NO: 5, 6, or 7.

21. An antigen-binding region according to claim 16, which comprises an L-CDR3 region depicted in SEQ ID NO: 13, 14, or 15.

22. An antigen-binding region according to claim 21, further comprising an L-CDR1 region depicted in SEQ ID NO: 13, 14, or 15.

23. An antigen-binding region according to claim 22, further comprising an L-CDR2 region depicted in SEQ ID NO: 13, 14, or 15.

24. An antigen-binding region according to claim 23, which comprises a variable light chain depicted in SEQ ID NO: 13, 14, or 15

25. An antigen-binding region according to claim 16, which comprises a heavy chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 5, 6, or 7; (ii) a sequence having at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 6, or 7; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 6, or 7.

26. An antigen-binding region according to claim 16, which comprises a light chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 13, 14, or 15; (ii) a sequence having at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 14, or 15; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 14, or 15.

27. An antibody to according to claims 15, which is an IgG.

28. An antibody to according to claim 27, which is an IgG1.

29. An isolated human antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of CD38, wherein the epitope comprises one or more amino acid residues of amino acid residues 1 to 215 of CD38 (SEQ ID NO: 22).

30. An isolated human antibody or functional fragment thereof of claim 29, wherein the epitope comprises one or more amino acid residues comprised in one or more of the amino acid stretches taken from the list of amino acid stretches 44-66, 82-94, 142-154, 148-164, 158-170, and 192-206 of CD38.

31. An antibody or functional fragment thereof according to claim 29, wherein said epitope is a linear epitope.

32. An antibody or functional fragment thereof according to claim 31, wherein said antigen-binding region comprises an H-CDR3 region depicted in SEQ ID NO: 6.

33. An antibody or functional fragment thereof according to claim 32, wherein said antigen-binding region further comprises an H-CDR2 region depicted in SEQ ID NO: 6.

34. An antibody or functional fragment thereof according to claim 33, wherein said antigen-binding region further comprises an H-CDR1 region depicted in SEQ ID NO: 6.

35. An antibody or functional fragment thereof according to claim 31, which comprises a variable heavy chain depicted in SEQ ID NO: 6.

36. An antibody or functional fragment thereof according to claim 31, wherein said antigen-binding region comprises an L-CDR3 region depicted in SEQ ID NO: 14.

37. An antibody or functional fragment thereof according to claim 36, wherein said antigen-binding region further comprises an L-CDR1 region depicted in SEQ ID NO: 14.

38. An antibody or functional fragment thereof according to claim 37, wherein said antigen-binding region further comprises an L-CDR2 region depicted in SEQ ID NO: 14.

39. An antibody or functional fragment thereof according to claim 31, which comprises a variable light chain depicted in SEQ ID NO: 14.

40. An antibody or functional fragment thereof according to claim 31, which comprises a heavy chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 6; (ii) a sequence having at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 6; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 6.

41. An antibody or functional fragment thereof according to claim 31, which comprises a light chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 14; (ii) a sequence having at least 60 percent

sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 14; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 14.

42. A functional fragment according to claim 31, which is a Fab or scFv antibody fragment.

43. An antibody according to claims 31, which is an IgG.

44. An antibody according to claim 43, which is an IgG1.

45. An antibody or functional fragment thereof according to claim 29, wherein said epitope is a conformational epitope.

46. An antibody or functional fragment thereof according to claim 45, wherein said antigen-binding region comprises an H-CDR3 region depicted in SEQ ID NO: 5, 7, or 8.

47. An antibody or functional fragment thereof according to claim 46, wherein said antigen-binding region further comprises an H-CDR2 region depicted in SEQ ID NO: 5, 7, or 8.

48. An antibody or functional fragment thereof according to claim 47, wherein said antigen-binding region further comprises an H-CDR1 region depicted in SEQ ID NO: 5, 7, or 8.

49. An antibody or functional fragment thereof according to claim 45, which comprises a variable heavy chain depicted in SEQ ID NO: 5, 7, or 8.

50. An antibody or functional fragment thereof according to claim 45, wherein said antigen-binding region comprises an L-CDR3 region depicted in SEQ ID NO: 13, 15, or 16.



51. An antibody or functional fragment thereof according to claim 50, wherein said antigen-binding region further comprises an L-CDR1 region depicted in SEQ ID NO: 13, 15, or 16.

52. An antibody or functional fragment thereof according to claim 51, wherein said antigen-binding region further comprises an L-CDR2 region depicted in SEQ ID NO: 13, 15, or 16.

53. An antibody or functional fragment thereof according to claim 45, which comprises a variable light chain depicted in SEQ ID NO: 13, 15, or 16.

54. An antibody or functional fragment thereof according to claim 45, which comprises a heavy chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 5, 7, or 8; (ii) a sequence having at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 7, or 8; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 5, 7, or 8.

55. An antibody or functional fragment thereof according to claim 45, which comprises a light chain amino acid sequence selected from the group consisting of (i) SEQ ID NO: 13, 15, or 16; (ii) a sequence having at least 60 percent sequence identity in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 15, or 16; and (iii) a sequence having at least 80 percent sequence homology in the CDR regions with the CDR regions depicted in SEQ ID NO: 13, 15, or 16.

56. A functional fragment according to claim 45, which is a Fab or scFv antibody fragment.

57. An antibody according to claims 45, which is an IgG.

58. An antibody according to claim 57, which is an IgG1.

59. A variable heavy chain of an isolated antibody or functional fragment thereof that is encoded by (i) a nucleic acid sequence comprising SEQ ID NO: 1, 2, 3, or 4, or (ii) a nucleic acid sequences that hybridizes under high stringency conditions to the complementary strand of SEQ ID NO: 1, 2, 3, or 4, wherein said antibody or functional fragment thereof is specific for an epitope of CD38.

60. A variable light chain of an isolated antibody or functional fragment thereof that is encoded by (i) a nucleic acid sequence comprising SEQ ID NO: 9, 10, 11, or 12, or (ii) a nucleic acid sequences that hybridizes under high stringency conditions to the complementary strand of SEQ ID NO: 9, 10, 11, or 12, wherein said antibody or functional fragment thereof is specific for an epitope of CD38.

61. An isolated nucleic acid sequence that encodes an antigen-binding region of a human antibody or functional fragment thereof that is specific for an epitope of CD38.

62. A nucleic acid sequence encoding a variable heavy chain of an isolated antibody or functional fragment thereof, which comprises (i) a sequence selected from the group consisting of SEQ ID NOS: 1, 2, 3 and 4 or (ii) a nucleic acid sequence that hybridizes under high stringency conditions to the complementary strand of SEQ ID NO: 1, 2, 3 or 4, wherein said antibody or functional fragment thereof is specific for an epitope of CD38.

63. A nucleic acid sequence encoding a variable light chain of an isolated antibody or functional fragment thereof, which comprises (i) a sequence selected from the group consisting of SEQ ID NOS: 9, 10, 11 and 12 or (ii) a nucleic acid sequence that hybridizes under high stringency conditions to the

complementary strand of SEQ ID NO: 9, 10, 11 or 12, wherein said antibody or functional fragment thereof is specific for an epitope of CD38.

64. A vector comprising a nucleic acid sequence according to any one of claims 61-63.

65. An isolated cell comprising a vector according to claim 64.

66. An isolated cell according to claim 65, wherein said cell is bacterial.

67. An isolated cell according to claim 65, wherein said cell is mammalian.

68. A pharmaceutical composition comprising an antibody or functional fragment according to any one of claims 1, 15 and 29 and a pharmaceutically acceptable carrier or excipient therefor.

69. A method for treating a disorder or condition associated with the undesired presence of CD38+ cells, comprising administering to a subject in need thereof an effective amount of the pharmaceutical composition according to claim 68.

70. A method according to claim 69, wherein said disorder or condition is a haematological disease.

71. A method according to claim 70 taken from the list of multiple myeloma, chronic lymphocytic leukemia, chronic myelogenous leukemia, acute myelogenous leukemia, and acute lymphocytic leukemia.

72. A method according to claim 69, wherein said disorder or condition is an inflammatory disease

73. A method according to claim 72 taken from the list of rheumatoid arthritis and systemic lupus erythematosus.

74. A method for targeting CD38+ cells in a subject or a cell sample, comprising the step of allowing said CD38+ cells to be contacted with an antibody or functional fragment thereof according to any one of claims 1, 15 and 29.

**ABSTRACT**

The present invention provides recombinant antigen-binding regions and antibodies and functional fragments containing such antigen-binding regions that are specific for CD38, which plays an integral role in various disorders or conditions. These antibodies, accordingly, can be used to treat, for example, hematological malignancies such as multiple myeloma. Antibodies of the invention also can be used in the diagnostics field, as well as for investigating the role of CD38 in the progression of disorders associated with malignancies. The invention also provides nucleic acid sequences encoding the foregoing antibodies, vectors containing the same, pharmaceutical compositions and kits with instructions for use.

**Figure 1a**

**Variable Heavy Chain DNA**

**3077\_VH1B (SEQ ID NO: 1):**

```
(1)  CAGGTGCAAT TGGTTCAGAG CGGCGCGGAA GTGAAAAAAC CGGGCGCGAG
(51)  CGTGAAAGTG AGCTGCAAAG CCTCCGGATA TACCTTTACT TCTTATTCTA
(101) TTAATTGGGT CCGCCAAGCC CCTGGGCAGG GTCTCGAGTG GATGGGCTAT
(151) ATCGATCCGA ATCGTGGCAA TACGAATTAC GCGCAGAAGT TTCAGGGCCG
(201) GGTGACCATG ACCCGTGATA CCAGCATTAG CACCGCGTAT ATGGAAGTGA
(251) GCAGCCTGCG TAGCGAAGAT ACGGCCGTGT ATTATTGCGC GCGTGAGTAT
(301) ATTTATTTTA TTCATGGTAT GCTTGATTTT TGGGGCCAAG GCACCCTGGT
(351) GACGGTTAGC TCA
```

**3079\_VH3 (SEQ ID NO: 2):**

```
(1)  CAGGTGCAAT TGGTGGAAG CGGCGGCGGC CTGGTGCAAC CGGGCGGCAG
(51)  CCTGCGTCTG AGCTGCGCGG CCTCCGATT TACCTTTTCT AATTATGGTA
(101) TGCAATTGGGT GCGCCAAGCC CCTGGGAAGG GTCTCGAGTG GGTGAGCAAT
(151) ATCCGTTCTG ATGGTAGCTG GACCTATTAT GCGGATAGCG TGAAAGGCCG
(201) TTTTACCATT TCACGTGATA ATTCGAAAAA CACCCTGTAT CTGCAAATGA
(251) ACAGCCTGCG TGCGGAAGAT ACGGCCGTGT ATTATTGCGC GCGTCGTTAT
(301) TGGTCTAAGT CTCATGCTTC TGTTACTGAT TATTGGGGCC AAGGCACCCCT
(351) GGTGACGGTT AGCTCA
```

**3080\_VH3 (SEQ ID NO: 3):**

```
(1)  CAGGTGCAAT TGGTGGAAG CGGCGGCGGC CTGGTGCAAC CGGGCGGCAG
(51)  CCTGCGTCTG AGCTGCGCGG CCTCCGATT TACCTTTTCT TCTTATGGTA
(101) TGCAATTGGGT GCGCCAAGCC CCTGGGAAGG GTCTCGAGTG GGTGAGCAAT
(151) ATCTATTCTG ATGGTAGCAA TACCTTTTAT GCGGATAGCG TGAAAGGCCG
(201) TTTTACCATT TCACGTGATA ATTCGAAAAA CACCCTGTAT CTGCAAATGA
(251) ACAGCCTGCG TGCGGAAGAT ACGGCCGTGT ATTATTGCGC GCGTAATATG
(301) TATCGTTGGC CTTTTCATTA TTTTTTTGAT TATTGGGGCC AAGGCACCCCT
(351) GGTGACGGTT AGCTCA
```

**3100\_VH 3 (SEQ ID NO: 4):**

```
(1)  CAGGTGCAAT TGGTGGAAG CGGCGGCGGC CTGGTGCAAC CGGGCGGCAG
(51)  CCTGCGTCTG AGCTGCGCGG CCTCCGATT TACCTTTTCT TCTAATGGTA
(101) TGTCTTGGGT GCGCCAAGCC CCTGGGAAGG GTCTCGAGTG GGTGAGCAAT
(151) ATCTCTTATC TTTCTAGCTC TACCTATTAT GCGGATAGCG TGAAAGGCCG
(201) TTTTACCATT TCACGTGATA ATTCGAAAAA CACCCTGTAT CTGCAAATGA
(251) ACAGCCTGCG TGCGGAAGAT ACGGCCGTGT ATTATTGCGC GCGTTTTTAT
(301) GGTTATTTTA ATTATGCTGA TGTTTGGGGC CAAGGCACCC TGGTGACGGT
(351) TAGCTCA
```

**3077\_1\_VH1B (SEQ ID NO: 31):**

```
(1)  CAGGTGCAAT TAGTCCAAAG TGGTGCGGAA GTGAAAAAAC CGGGCGCGAG
(51)  CGTGAAAGTG AGCTGCAAAG CCTCCGGATA TACCTTTACT TCTTATTCTA
(101) TTAATTGGGT CCGCCAAGCC CCTGGGCAGG GTCTCGAGTG GATGGGCTAT
(151) ATCGATCCGA ATCGTGGCAA TACGAATTAC GCGCAGAAGT TTCAGGGCCG
(201) GGTGACCATG ACCCGTGATA CCAGCATTAG CACCGCGTAT ATGGAAGTGA
(251) GCAGCCTGCG TAGCGAAGAT ACGGCCGTGT ATTATTGCGC GCGTGAGTAT
```

Michael TESAR  
"Anti-CD38 Human Antibodies and Uses Thereof"  
Attorney Docket No. 37629-0085C

(301) ATTTATTTTA TTCATGGTAT GCTTGATTTT TGGGGCCAAG GCACCCTGGT  
(351) GACGGTTAGC TCA

**Figure 1b**

**Variable Heavy Chain Peptide**

(CDR Regions in **Bold**)

**3077\_VH1B (SEQ ID NO: 5):**

(1) QVQLVQSGAE VKKPGASVKV SCKASGYTFT **SYSINWVRQA** PGQGLEWMGY  
(51) IDPNRGNTNY **AQKFQGRVTM** TRDTSISTAY MELSSLRSED TAVYYCAREY  
(101) **IYFIHGMLDF** WGQGTTLVTVS S

**3079\_VH3 (SEQ ID NO: 6):**

(1) QVQLVESGGG LVQPGGSLRL SCAASGFTFS **NYGMHWVRQA** PGKGLEWVSN  
(51) IRSDGSWTTY **ADSVKGRFTI** SRDNSKNTLY LQMNSLRAED TAVYYCARRY  
(101) **WSKSHASVTD** YWGQGTTLVTV SS

**3080\_VH3 (SEQ ID NO: 7):**

(1) QVQLVESGGG LVQPGGSLRL SCAASGFTFS **SYGMHWVRQA** PGKGLEWVSN  
(51) **IYSDGSNTFY** **ADSVKGRFTI** SRDNSKNTLY LQMNSLRAED TAVYYCARNM  
(101) **YRWPFHYFFD** YWGQGTTLVTV SS

**3100\_VH 3 (SEQ ID NO: 8):**

(1) QVQLVESGGG LVQPGGSLRL SCAASGFTFS **SNGMSWVRQA** PGKGLEWVSN  
(51) **ISYLSSSTYY** **ADSVKGRFTI** SRDNSKNTLY LQMNSLRAED TAVYYCARFY  
(101) **GYFNYADVWG** QGTTLVTVSS



**Figure 2a**

**Variable Light Chain DNA**

**3077\_Vk kappa 2 (SEQ ID NO: 9):**

```
(1)  GATATCGTGA TGACCCAGAG CCCACTGAGC CTGCCAGTGA CTCCGGGCGA
(51) GCCTGCGAGC ATTAGCTGCA GAAGCAGCCA AAGCCTGCTT TTTATTGATG
(101) GCAATAATTA TCTGAATTGG TACCTTCAAA AACCAGGTCA AAGCCCCGAG
(151) CTATTAATTT ATCTTGGTTC TAATCGTGCC AGTGGGGTCC CGGATCGTTT
(201) TAGCGGCTCT GGATCCGGCA CCGATTTTAC CCTGAAAATT AGCCGTGTGG
(251) AAGCTGAAGA CGTGGGCGTG TATTATTGCC AGCAGTATTC TTCTAAGTCT
(301) GCTACCTTTG GCCAGGGTAC GAAAGTTGAA ATTAAACGTA CG
```

**3079\_Vk kappa 1 (SEQ ID NO: 10):**

```
(1)  GATATCCAGA TGACCCAGAG CCCGTCTAGC CTGAGCGCGA GCGTGGGTGA
(51) TCGTGTGACC ATTACCTGCA GAGCGAGCCA GGATATTTCT GCTTTTCTGA
(101) ATTGGTACCA GCAGAAACCA GGTAAAGCAC CGAAACTATT AATTTATAAG
(151) GTTCTAATT TGCAAAGCGG GGTCCCGTCC CGTTTTAGCG GCTCTGGATC
(201) CGGCACTGAT TTTACCTTGA CCATTAGCAG CCTGCAACCT GAAGACTTTG
(251) CGACTTATTA TTGCCAGCAG GCTTATTCTG GTTCTATTAC CTTTGGCCAG
(301) GGTACGAAAG TTGAAATTAA ACGTACG
```

**3080\_VI lambda 3 (SEQ ID NO: 11):**

```
(1)  GATATCGAAC TGACCCAGCC GCCTTCAGTG AGCGTTGCAC CAGGTCAGAC
(51) CGCGCGTATC TCGTGTAGCG GCGATAATAT TGGTAATAAG TATGTTTCTT
(101) GGTACCAGCA GAAACCCGGG CAGGCGCCAG TTGTTGTGAT TTATGGTGAT
(151) AATAATCGTC CCTCAGGCAT CCCGGAACGC TTTAGCGGAT CCAACAGCGG
(201) CAACACCGCG ACCCTGACCA TTAGCGGCAC TCAGGCGGAA GACGAAGCGG
(251) ATTATTATTG CTCTTCTTAT GATTCTTCTT ATTTTGTGTT TGGCGGCGGC
(301) ACGAAGTTAA CCGTTCTTGG CCAG
```

**3100\_VI lambda 3 (SEQ ID NO: 12):**

```
(1)  GATATCGAAC TGACCCAGCC GCCTTCAGTG AGCGTTGCAC CAGGTCAGAC
(51) CGCGCGTATC TCGTGTAGCG GCGATAATAT TGGTCATTAT TATGCTTCTT
(101) GGTACCAGCA GAAACCCGGG CAGGCGCCAG TTCTTGTGAT TTATCGTGAT
(151) AATGATCGTC CCTCAGGCAT CCCGGAACGC TTTAGCGGAT CCAACAGCGG
(201) CAACACCGCG ACCCTGACCA TTAGCGGCAC TCAGGCGGAA GACGAAGCGG
(251) ATTATTATTG CCAGTCTTAT GATTATCTTC ATGATTTTGT GTTTGGCGGC
(301) GGCACGAAGT TAACCGTTCT TGGCCAG
```

**Figure 2b**

**Variable Light Chain Peptide**

(CDR Regions in **Bold**)

**3077\_Vk kappa 2 (SEQ ID NO: 13):**

(1) DIVMTQSPLS LPVTPGEPAS ISCRSSQSLL **FIDGNNYLNW** YLQKPGQSPQ  
(51) **LLIYLGSNRA** SGVPDRFSGS GSGTDFTLKI SRVEAEDGVV **YYCQYSSKS**  
(101) ATFGQGTKVE IKRT

**3079\_Vk kappa 1 (SEQ ID NO: 14):**

(1) DIQMTQSPSS LSASVGDRVT ITCRASQDIS **AFLNWFQQKP** GKAPKLLIYK  
(51) **VSNLQSGVPS** RFGSGSGTD FTLTISSLPQ EDFATYYCQ **AYSGSITFGQ**  
(101) GTKVEIKRT

**3080\_VI lambda 3 (SEQ ID NO: 15):**

(1) DIELTQPPSV SVAPGQTARI SCSGDNIGNK **YVSWYQQKPG** QAPVVVIYGD  
(51) **NNRPSGIPER** FSGSNSGNTA TLTISGTQAE DEADYYCSSY **DSSYFVFGGG**  
(101) TKLTVLGQ

**3100\_VI lambda 3 (SEQ ID NO: 16):**

(1) DIELTQPPSV SVAPGQTARI SCSGDNIGHY **YASWYQQKPG** QAPVLVIYRD  
(51) **NDRPSGIPER** FSGSNSGNTA TLTISGTQAE DEADYYCQSY **DYLHDFVFGG**  
(101) GTKLTVLGQ

**Figure 3**

**Variable Heavy Chain Consensus Sequences**

(CDR Regions in **Bold**)

**VH1B Consensus (SEQ ID NO: 17):**

(1) QVQLVQSGAE VKKPGASVKV SCKASGYTFT **SYMHWVRQA** PGQGLEWMGW  
(51) **INPNSGGTNY AQKFQGRVTM** TRDTSISTAY MELSSLRSED TAVYYCARWG  
(101) **GDGFYAMDYW GQGLVTVSS**

**VH3 Consensus (SEQ ID NO: 18):**

(1) QVQLVESGGG LVQPGGSLRL SCAASGFTFS **SYAMSWVRQA** PGKGLEWVSA  
(51) **ISGSGGSTYY ADSVKGRFTI** SRDNSKNTLY LQMNSLRAED TAVYYCARWG  
(101) **GDGFYAMDYW GQGLVTVS S**

Figure 4

**Variable Light Chain Consensus Sequences**

(CDR Regions in **Bold**)

**VL<sub>λ3</sub> Consensus (SEQ ID NO: 19):**

(1) SYELTQPPSV SVAPGQTARI **SCSGDALGDK YASWYQQKPG QAPVLVIYDD**  
(51) **SDRPSGIPER** FSGSNSGNTA TLTISGTQAE DEADYYC**QQH YTT**PPVFGGG  
(101) TKLTVLG

**VL<sub>k1</sub> Consensus (SEQ ID NO: 20):**

(1) DIQMTQSPSS LSASVGDRVIT ITCRAS**QGIS SYLAWYQQKP GKAPKLLIYA**  
(51) **ASSLQSGVPS** RFSGSGSGTD FTLTISSLQP EDFATYYC**QQ HY**TTTPPTFGQ  
(101) GTKVEIKR

**VL<sub>k2</sub> Consensus (SEQ ID NO: 21):**

(1) DIVMTQSPLS LPVTPGEPAS ISCRSS**QSL H**SNNGYNYLDW YLQKPGQSPQ  
(51) **LLIYLGSNRA** SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**QQHY**TTTP  
(101) PTFGQGTKVE IKR

**Figure 5**

**Peptide Sequence of CD38**

(SEQ ID NO: 22):

```
1    mancefspvs gdkpccrlsr raqlclgysi lvlilvvvla vvvprwrqqw sgpgttkrfp
61   etvlarcvky teihpemrhv dcqsvwdafk gafiskhpcn iteedyqplm klgtqtvpcn
121  killwsrikd lahqftqvqr dmftledtll gyladdltwc gefntskiny qscpdwrkdc
181  snnpvsfvwk tvsrrfaaaa cdvvhvmIng srskifdkns tfgsvevhn1 qpekvtlea
241  wvihggreds rdlcqdptik elesiiskrn iqfsckniyr pdkflqcvkn pedssctsei
```

**Figure 6**

**Nucleotide Sequence of Chimeric OKT10**

**Heavy Chain (SEQ ID NO: 23):**

cagggtggaat tgggtggaatc tggaggatcc ctgaaactct cctgtgcagc ctcaggattc  
gatttttagta gatcctggat gaattgggtc cggcaggctc caggaaaagg gctagaatgg  
attggagaaa ttaatccaga tagcagtacg ataaactata cgacatctct aaaggataaa  
ttcatcatct ccagagacaa cgccaaaaat acgctgtacc tgcaaatgac caaagtgaga  
tctgaggaca cagcccttta ttactgtgca agatatggta actggtttcc ttattggggc  
caagggactc tgggtcaactgt cagctcagcc tccaccaagg gtccatcggc cttccccctg  
gcaccctcct ccaagagcac ctctgggggc acagcggccc tgggctgcct ggtcaaggac  
tacttccccg aaccggtgac ggtgtcgtgg aactcaggcg cctgaccag cggcgtgcac  
accttccccg ctgtcctaca gtctcagga ctctactccc tcagcagcgt ggtgaccgtg  
ccctccagca gcttgggcac ccagacctac atctgcaacg tgaatcaca gccagcaac  
accaaggtgg acaagaaagt tgagcccaa tcttgtgaca aaactcacac atgcccaccg  
tgcccagcac ctgaactcct ggggggaccg tcagtcttcc ttttcccccc aaaacccaag  
gacaccctca tgatctcccg gaccctgag gtcacatgcg tgggtggtgga cgtgagccac  
gaagaccctg aggtcaagtt caactggtac gtggacggcg tggaggtgca taatgccaa  
acaaagccgc gggaggagca gtacaacagc acgtaccggg tggtcagcgt cctcacgcgc  
ctgaccagg actggctgaa tggcaaggag tacaagtgca aggtctccaa caaagccctc  
ccagccccc tgcagaaaac catctccaaa gccaaagggc agccccgaga accacagggtg  
tacaccctgc ccccatcccg ggatgagctg accaagaacc aggtcagcct gacctgctg  
gtcaaaggct tctatcccag cgacatcgcc gtggagtggg agagcaatgg gcagccggag  
aacaactaca agaccacgcc tcccgtgctg gactccgacg gctccttctt cctctacagc  
aagctcaccg tggacaagag cagggtggcag caggggaacg tcttctcatg ctccgtgatg  
catgaggctc tgcacaacca ctacacgcag aagagcctct ccctgtctcc gggtaaa

**Light Chain (SEQ ID NO: 24):**

gatatcctga tgaccagtc tcaaaaaatc atgccacat cagtgggaga cagggtcagc  
gtcacctgca aggcagtc aaatgtggat actaatgtag cctggatatca acagaaacca

Michael TESAR  
"Anti-CD38 Human Antibodies and Uses Thereof"  
Attorney Docket No. 37629-0085C

ggacagtctc ctaaagcact gatttactcg gcacccctacc gatacagtgg agtccctgat  
cgcttcacag gcagtggatc tgggacagat ttcactctca ccatcaccaa tgtgcagtct  
gaggacttgg cagagtatct ctgtcagcaa tatgacagct atcctctcac gttcgggtgct  
gggaccaagc tggacctgaa acgtacgggtg gctgcaccat ctgtcttcat cttcccgcca  
tctgatgagc agttgaaatc tgggaactgcc tctgttgtgt gcctgctgaa taacttctat  
cccagagagg ccaaagtaca gtggaagggtg gataacgccc tccaatcggg taactcccag  
gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg  
ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc  
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gt

Fig.7: Schematic Overview of Epitopes

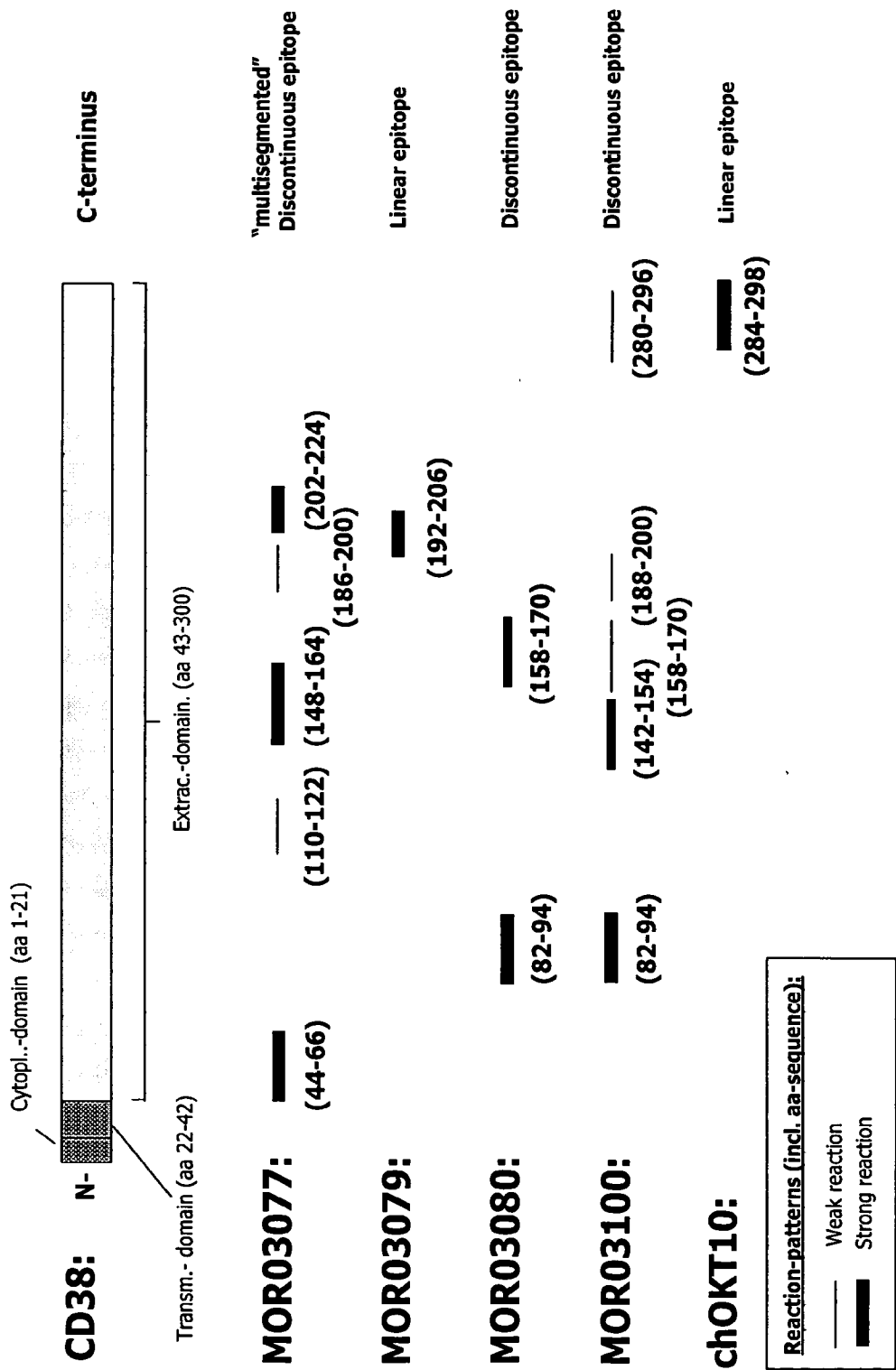




Figure 8: DNA sequence of pMOPRH@\_h\_IgG1\_1

```

                                StyI
                                ~~~~~
601   TCGCTATTAC CATGGTGTATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA
      AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

                                AatII
                                ~~~~~
651   TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
      ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

701   TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
      ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

751   ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
      TGTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

801   GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA CTGCTTACTG
      CAGATATATT CGTCTCGAGA GACCGATTGA TCTCTGGGT GACGAATGAC

                                pMORPH®_Ig_FOR 100.0%
                                ~~~~~
851   GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC
      CGAATAGCTT TAATTATGCT GAGTGATATC CCTCTGGGT CGACCGATCG

      M K H L W F F L L L V A A P R
901   GCCACCATGA AACACCTGTG GTTCTTCCTC CTGCTGGTGG CAGCTCCCAG
      CGGTGGTACT TTGTGGACAC CAAGAAGGAG GACGACCACC GTCGAGGGTC

                                EcoRI
                                ~~~~~
                                BlnI
                                ~~~~~
                                StyI
                                ~~~~~
      W V L S Q V E F C R R L A Q
951   ATGGGTCCTG TCCCAGGTGG AATTCTGCAG GCGGTTAGCT CAGCCTCCAC
      TACCCAGGAC AGGGTCCACC TTAAGACGTC CGCCAATCGA GTCGGAGGTG

      A S T

StyI
-----
      K G P S V F P L A P S S K S T S G
1001  CAAGGGTCCA TCGGTCTTCC CCCTGGCACC CTCCTCCAAG AGCACCTCTG
      GTTCCCAGGT AGCCAGAAGG GGGACCGTGG GAGGAGGTTC TCGTGGAGAC

      G T A A L G C L V K D Y F P E P
1051  GGGGCACAGC GGCCCTGGGC TGCCTGGTCA AGGACTACTT CCCCGAACCG
      CCCCCTGTCTG CCGGGACCCG ACGGACCAGT TCCTGATGAA GGGGCTTGGC
  
```

Michael TESAR  
"Anti-CD38 Human Antibodies and Uses Thereof"  
Attorney Docket No. 37629-0085C

1101 V T V S W N S G A L T S G V H T F .  
GTGACGGTGT CGTGGAAGTC AGGCGCCCTG ACCAGCGGCG TGCACACCTT  
CACTGCCACA GCACCTTGAG TCCGCGGGAC TGGTCGCCGC ACGTGTGGAA

1151 P A V L Q S S G L Y S L S S V V T .  
CCCGGCTGTC CTACAGTCCT CAGGACTCTA CTCCCTCAGC AGCGTGGTGA  
GGGCCGACAG GATGTCAGGA GTCCTGAGAT GAGGGAGTCG TCGCACCCT

1201 V P S S S L G T Q T Y I C N V N  
CCGTGCCCTC CAGCAGCTTG GGCACCCAGA CCTACATCTG CAACGTGAAT  
GGCACGGGAG GTCGTCGAAC CCGTGGGTCT GGATGTAGAC GTTGCACTTA

StyI  
~~~~~

1251 H K P S N T K V D K K V E P K S C .  
CACAAGCCCA GCAACACCAA GGTGGACAAG AAAGTTGAGC CCAAATCTTG  
GTGTTCCGGT CGTTGTGGTT CCACCTGTTT TTTCAACTCG GGTTTAGAAC

1301 D K T H T C P P C P A P E L L G G .  
TGACAAAAC CACACATGCC CACCGTGCCC AGCACCTGAA CTCCTGGGGG  
ACTGTTTTGA GTGTGTACGG GTGGCACGGG TCGTGACTT GAGGACCCCC

BbsI StyI  
~~~~~

1351 P S V F L F P P K P K D T L M I  
GACCGTCAGT CTTCTCTTTC CCCCCAAAAC CCAAGGACAC CCTCATGATC  
CTGGCAGTCA GAAGGAGAAG GGGGGTTTTG GGTTCCTGTG GGAGTACTAG

BbsI  
~~~~~

1401 S R T P E V T C V V V D V S H E D .  
TCCCGGACCC CTGAGGTCAC ATGCGTGGTG GTGGACGTGA GCCACGAAGA  
AGGGCCTGGG GACTCCAGTG TACGCACCAC CACCTGCACT CGGTGCTTCT

BbsI  
~

1451 P E V K F N W Y V D G V E V H N A .  
CCCTGAGGTC AAGTTCAACT GGTACGTGGA CGGCGTGGAG GTGCATAATG  
GGGACTCCAG TTCAAGTTGA CCATGCACCT GCCGCACCTC CACGTATTAC

1501 K T K P R E E Q Y N S T Y R V V  
CCAAGACAAA GCCGCGGGAG GAGCAGTACA ACAGCACGTA CCGGGTGGTC  
GGTTCTGTTT CGGCGCCCTC CTCGTCATGT TGTCGTGCAT GGCCACCAG

1551 S V L T V L H Q D W L N G K E Y K .  
AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA AGGAGTACAA  
TCGCAGGAGT GGCAGGACGT GGTCTGACC GACTTACCGT TCCTCATGTT

1601 C K V S N K A L P A P I E K T I S .  
GTGCAAGGTC TCCAACAAAG CCCTCCCAGC CCCCATCGAG AAAACCATCT  
CACGTTCCAG AGGTTGTTTC GGGAGGGTCG GGGGTAGCTC TTTTGGTAGA

BsrGI  
~~~~~

1651 K A K G Q P R E P Q V Y T L P P  
CCAAAGCCAA AGGGCAGCCC CGAGAACCCAG AGGTGTACAC CCTGCCCCCA  
GGTTTCGGTT TCCCGTCGGG GCTCTTGGTG TCCACATGTG GGACGGGGGT

Michael TESAR  
"Anti-CD38 Human Antibodies and Uses Thereof"  
Attorney Docket No. 37629-0085C

1701 S R D E L T K N Q V S L T C L V K .  
TCCCGGGATG AGCTGACCAA GAACCAGGTC AGCCTGACCT GCCTGGTCAA  
AGGGCCCTAC TCGACTGGTT CTTGGTCCAG TCGGACTGGA CGGACCAGTT

1751 - G F Y P S D I A V E W E S N G Q P .  
AGGCTTCTAT CCCAGCGACA TCGCCGTGGA GTGGGAGAGC AATGGGCAGC  
TCCGAAGATA GGGTCGCTGT AGCGGCACCT CACCCTCTCG TTACCCGTCTG

1801 - E N N Y K T T P P V L D S D G S  
CGGAGAACAA CTACAAGACC ACGCCTCCCG TGCTGGACTC CGACGGCTCC  
GCCTCTTGTT GATGTTCTGG TGCGGAGGGC ACGACCTGAG GCTGCCGAGG

1851 F F L Y S K L T V D K S R W Q Q G .  
TTCTTCCTCT ACAGCAAGCT CACCGTGGAC AAGAGCAGGT GGCAGCAGGG  
AAGAAGGAGA TGTCGTTCTGA GTGGCACCTG TTCTCGTCCA CCGTCGTCCC

BbsI NsiI  
~~~~~  
1901 - N V F S C S V M H E A L H N H Y T .  
GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCCTACA  
CTTGCAGAAG AGTACGAGGC ACTACGTACT CCGAGACGTG TTGGTGATGT

SapI PmeI  
~~~~~  
1951 - Q K S L S L S P G K \*  
CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA AATGAGGGCC CGTTTAAACC  
GCGTCTTCTC GGAGAGGGAC AGAGGCCCAT TTAATCCCGG GCAAATTTGG

2001 CGCTGATCAG CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG  
GCGACTAGTC GGAGCTGACA CGGAAGATCA ACGGTCCGTA GACAACAAAC

~~~~~  
pMORPH<sup>®</sup>\_Ig\_REV 100.0%  
2051 CCCCTCCCCC GTGCCTTCCT TGACCCTGGA AGGTGCCACT CCCACTGTCC  
GGGGAGGGGG CACGGAAGGA ACTGGGACCT TCCACGGTGA GGGTGACAGG

Figure 9: DNA Sequence of Ig kappy light chain expression vector pMORPH@\_h\_Igk\_1

```

                StyI
                ~~~~~
601  TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA
    AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

651  TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
    ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

701  TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
    ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

751  ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
    TGTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

801  GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA CTGCTTACTG
    CAGATATATT CGTCTCGAGA GACCGATTGA TCTCTTGGGT GACGAATGAC

                pMORPH®_Ig_FOR 100%
                ~~~~~
851  GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC
    CGAATAGCTT TAATTATGCT GAGTGATATC CCTCTGGGTT CGACCGATCG

+1      M  V  L  Q  T  Q  V  F  I  S  L  L  L  W  I
      StyI
      ~~~~~
901  GCCACCATGG TGTTCAGAC CCAGGTCTTC ATTTCTCTGT TGCTCTGGAT
    CGGTGGTACC ACAACGTCTG GGTCCAGAAG TAAAGAGACA ACGAGACCTA
                BbsI
                ~~~~~

+1      S  G  A  Y  G  D  I  V  M  I  K  R  T  V  A  A
      EcoRV
      ~~~~~
      BsiWI
      ~~~~~
951  CTCTGGTGCC TACGGGGATA TCGTGATGAT TAAACGTACG GTGGCTGCAC
    GAGACCACGG ATGCCCTAT AGCACTACTA ATTTGCATGC CACCGACGTG

+1  P  S  V  F  I  F  P  P  S  D  E  Q  L  K  S  G  T
1001 CATCTGTCTT CATCTTCCCG CCATCTGATG AGCAGTTGAA ATCTGGAAC
    GTAGACAGAA GTAGAAGGGC GGTAGACTAC TCGTCAACTT TAGACCTTGA
      BbsI
      ~~~~~
```

Michael TESAR  
"Anti-CD38 Human Antibodies and Uses Thereof"  
Attorney Docket No. 37629-0085C

```
+1  A S V V C L L N N F Y P R E A K V
1051 GCCTCTGTTG TGTGCCTGCT GAATAACTTC TATCCCAGAG AGGCCAAAGT
    CCGAGACAAC ACACGACGA CTTATTGAAG ATAGGGTCTC TCCGGTTTCA

+1  Q W K V D N A L Q S G N S Q E S
1101 ACAGTGGAAG GTGGATAACG CCCTCCAATC GGGTAACTCC CAGGAGAGTG
    TGTCACCTTC CACCTATTGC GGGAGGTTAG CCCATTGAGG GTCCTCTCAC

+1  V T E Q D S K D S T Y S L S S T L
1151 TCACAGAGCA GGACAGCAAG GACAGCACCT ACAGCCTCAG CAGCACCTTG
    AGTGTCTCGT CCTGTCGTTC CTGTCGTGGA TGTCGGAGTC GTCGTGGGAC

+1  T L S K A D Y E K H K V Y A C E V
    BlpI
-----
1201 ACGCTGAGCA AAGCAGACTA CGAGAAACAC AAAGTCTACG CCTGCGAAGT
    TGCGACTCGT TTCGTCTGAT GCTCTTTGTG TTTCAGATGC GGACGCTTCA

+1  T H Q G L S S P V T K S F N R G
1251 CACCCATCAG GGCCTGAGCT CGCCCGTCAC AAAGAGCTTC AACAGGGGAG
    GTGGGTAGTC CCGGACTCGA GCGGGCAGTG TTTCTCGAAG TTGTCCCCTC

+1  E C *
                                PmeI                                pMORPHo_Ig_REV 100%
                                ~~~~~                                =====
1301 AGTGTTAGGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT
    TCACAATCCC CGGGCAAATT TGGGCGACTA GTCGGAGCTG ACACGGAAGA

=
1351 AGTTGCCAGC CATCTGTTGT TTGCCCCTCC CCCGTGCCTT CCTTGACCCT
    TCAACGGTCG GTAGACAACA AACGGGGAGG GGGCACGGAA GGAAGTGGGA
```

**Figure 10: DNA Sequence of HuCAL<sup>®</sup> Ig lambda light chain vector pMORPH<sup>®</sup>\_h\_Igλ\_1**

```

                StyI
                ~~~~~
601  TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA
    AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

651  TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
    ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

701  TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
    ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

751  ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
    TGTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

801  GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA CTGCTTACTG
    CAGATATATT CGTCTCGAGA GACCGATTGA TCTCTTGGGT GACGAATGAC

                pM_Ig_FOR 100.0%                NheI
                =====                ~~~~~
851  GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC
    CGAATAGCTT TAATTATGCT GAGTGATATC CCTCTGGGTT CGACCGATCG

+1      M  A  W  A  L  L  L  L  T  L  L  T  Q  G  T
      StyI
      ~~~~~
901  GCCACCATGG CCTGGGCTCT GCTGCTCCTC ACCCTCCTCA CTCAGGGCAC
    CGGTGGTACC GGACCCGAGA CGACGAGGAG TGGGAGGAGT GAGTCCCGTG

+2      T  V  L  G  Q
+1      G  S  W  A  D  I  V  M  H  E  V
      BamHI      EcoRV      HpaI      StyI
      ~~~~~      ~~~~~      ~~~~~      ~~~~~
951  AGGATCCTGG GCTGATATCG TGATGCACGA AGTTAACCGT CCTAGGTCAG
    TCCTAGGACC CGACTATAGC ACTACGTGCT TCAATTGGCA GGATCCAGTC

+2      P  K  A  A  P  S  V  T  L  F  P  P  S  S  E  E  L
      StyI
      ~~~~~
1001  CCCAAGGCTG CCCCTCGGT CACTCTGTTC CCGCCCTCCT CTGAGGAGCT
    GGGTTCCGAC GGGGGAGCCA GTGAGACAAG GGCGGGAGGA GACTCCTCGA

+2      Q  A  N  K  A  T  L  V  C  L  I  S  D  F  Y  P
1051  TCAAGCCAAC AAGGCCACAC TGGTGTGTCT CATAAGTGAC TTCTACCCGG
    AGTTCGGTTG TTCCGGTGTG ACCACACAGA GTATTCACTG AAGATGGGCC

```

Michael TESAR  
"Anti-CD38 Human Antibodies and Uses Thereof"  
Attorney Docket No. 37629-0085C

+2 G A V T V A W K G D S S P V K A G  
1101 GAGCCGTGAC AGTGGCCTGG AAGGGAGATA GCAGCCCCGT CAAGGCGGGA  
CTCGGCACTG TCACCGGACC TTCCCTCTAT CGTCGGGGCA GTTCCGCCCT

+2 V E T T T P S K Q S N N K Y A A S  
1151 GTGGAGACCA CCACACCTC CAAACAAAGC AACAACAAGT ACGCGGCCAG  
CACCTCTGGT GGTGTGGGAG GTTTGTTTCG TTGTTGTTCA TGCGCCGGTC

+2 S Y L S L T P E Q W K S H R S Y  
1201 CAGCTATCTG AGCCTGACGC CTGAGCAGTG GAAGTCCCAC AGAAGCTACA  
GTCGATAGAC TCGGACTGCG GACTCGTCAC CTTCAGGGTG TCTTCGATGT

+2 S C Q V T H E G S T V E K T V A P  
BbsI  
~~~~~

1251 GCTGCCAGGT CACGCATGAA GGGAGCACCG TGGAGAAGAC AGTGGCCCCCT  
CGACGGTCCA GTGCGTACTT CCCTCGTGGC ACCTCTTCTG TCACCGGGGA

+2 T E C S \*

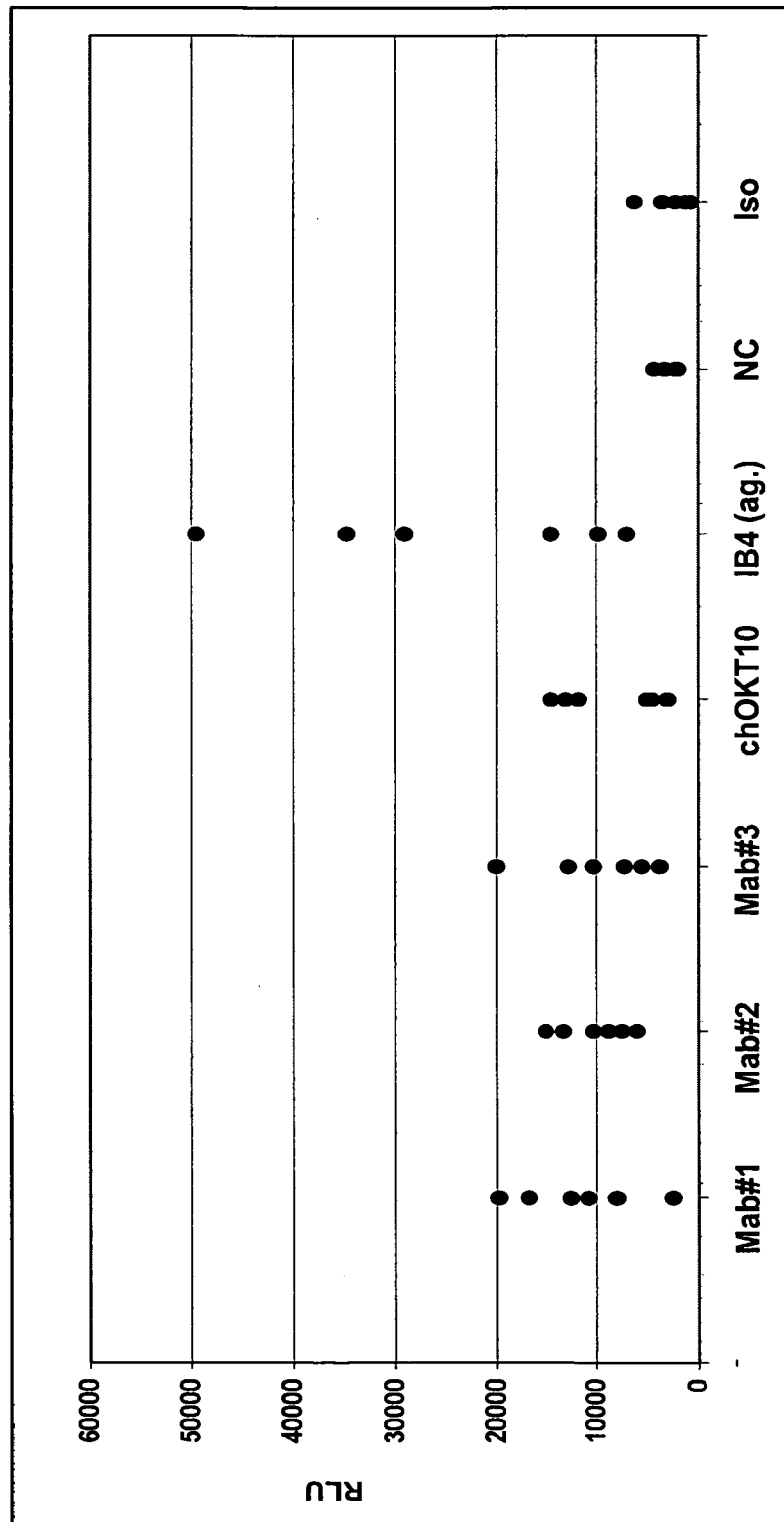
PmeI  
~~~~~

1301 ACAGAATGFT CATAGGGGCC CGTTTAAACC CGCTGATCAG CCTCGACTGT  
TGTCTTACAA GTATCCCCGG GCAAATTTGG GCGACTAGTC GGAGCTGACA

pM\_Ig\_REV 100%

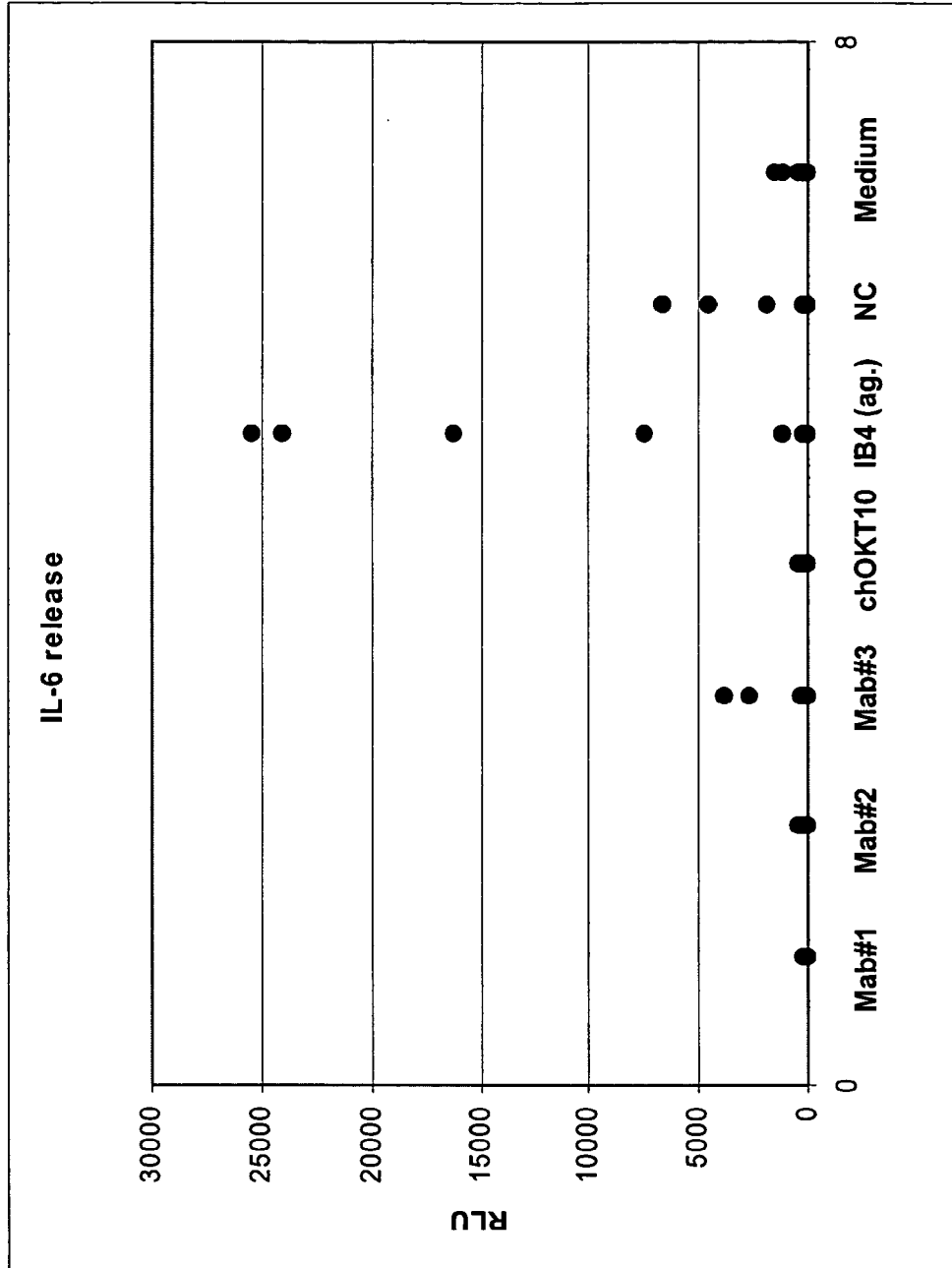
=====

1351 GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC GTGCCTTCCT  
CGGAAGATCA ACGGTCGGTA GACAACAAAC GGGGAGGGGG CACGGAAGGA  
pM\_Ig\_REV 100.0%  
=====

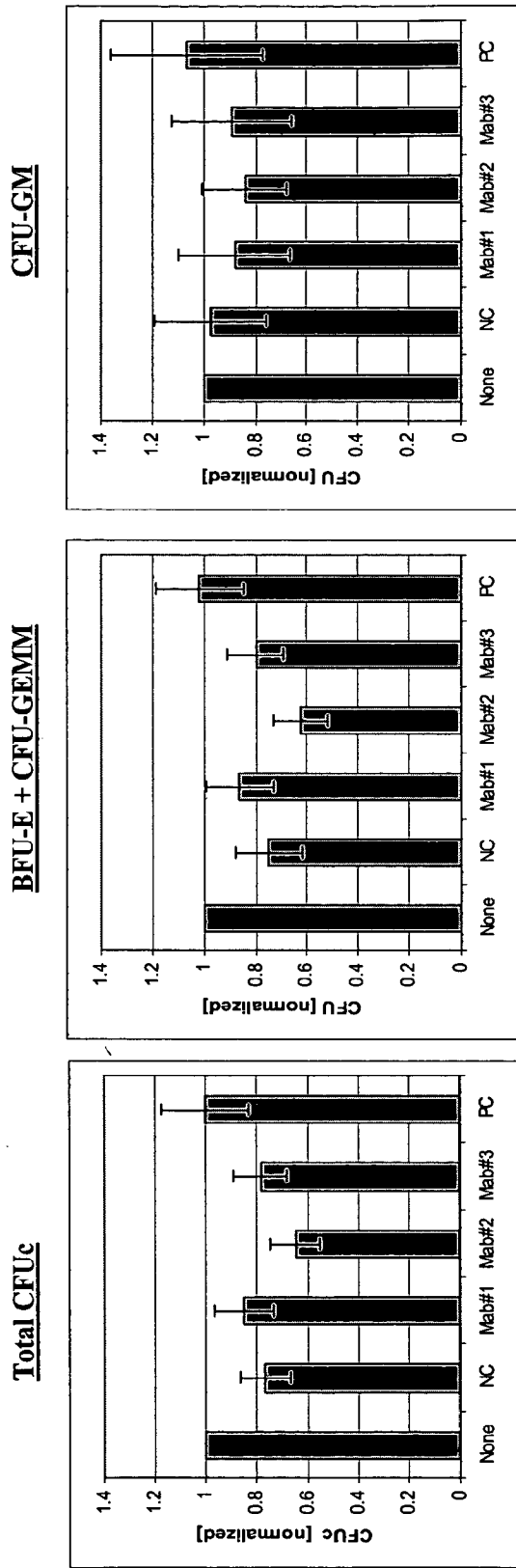




**Fig. 12: IL-6 Release Assay**



**Fig. 13: Cytotoxicity towards CD34+/CD38+ progenitor cells**



**Fig. 14: ADCC with different cell-lines**

| Cell line          | Culture Collection              | Origin           | Expression [MFI]    | Max. specific killing [%] in ADCC <sup>ac</sup> |       |       |      |
|--------------------|---------------------------------|------------------|---------------------|-------------------------------------------------|-------|-------|------|
|                    |                                 |                  |                     | Mab#1                                           | Mab#2 | Mab#3 | PC   |
| RPMI 8226          | ATCC CCL-155                    | MM               | 405.71              | 56                                              | 58    | 54    | 46   |
| KMS-12-BM          | DSMZ ACC551                     | MM               | 142.29              | 26                                              | 32    | 30    | 34   |
| NCI-H929           | ECACC95050415                   | MM               | 45.01               | 68                                              | 73    | 38    | 54   |
| OPM-2              | DSMZ ACC50                      | MM               | 37.99               | 6                                               | 13    | 3     | 7    |
| U-266              | ECACC85051003                   | MM               | 26.14               | 17                                              | 14    | 12    | 16   |
| KMS-11             | Namba et al., 1989 <sup>b</sup> | MM               | 26.81 <sup>d</sup>  | 22                                              | 30    | 26    | 28   |
| JVM-13             | DSMZACC19                       | CLL              | 463.93              | 11                                              | 20    | 12    | 15   |
| JVM-2              | DSMZACC12                       | CLL              | 140.84              | 22                                              | 28    | 10    | 24   |
| CCRF-CEM           | ECACC85112105                   | ALL              | 301.46              | 24                                              | 29    | 20    | 22   |
| Jurkat             | DSMZ ACC282                     | ALL              | 202.99              | 7                                               | 8     | 13    | 12   |
| AML-193            | DSMZ ACC549                     | AML              | 62.69 <sup>d</sup>  | 33                                              | 26    | 39    | 33   |
| OCI-AML5           | DSMZ ACC247                     | AML              | 207.55 <sup>d</sup> | 20                                              | 21    | 16    | 26   |
| NB-4               | DSMZ ACC207                     | AML              | 164.7 <sup>d</sup>  | 36                                              | 38    | 32    | 37   |
| THP-1              | DSMZ ACC16                      | AML              | 34.41               | 64                                              | 59    | 38    | 43   |
| HL-60 <sup>d</sup> | DSMZ ACC3                       | AML              | 18.43 <sup>d</sup>  | 29                                              | 35    | 29    | 29   |
| Raji               | Burkitt's Lymph.                | Burkitt's lymph. | n.d.                | 53                                              | 62    | 48    | n.d. |

**Fig. 15: ADCC with MM-samples**

| Antibodies<br>Parameters:            | Mab#1       | Mab#2       | Mab#3       | PC          |
|--------------------------------------|-------------|-------------|-------------|-------------|
|                                      |             |             |             |             |
| MM samples: EC50 [nM] <sup>a</sup> : | 0.116-0.202 | 0.006-0.185 | 0.027-0.249 | 0.282-0.356 |
| MM samples: Max spec. killing [%]    | 13.1 - 61.6 | 16.2 - 57.9 | 13.6 - 36.0 | 15.5 - 49.5 |

# **Application Data Sheet**

## **Application Information**

|                                  |                                              |
|----------------------------------|----------------------------------------------|
| Application number::             | Unassigned                                   |
| Filing Date::                    | August 6, 2004                               |
| Application Type::               | Provisional                                  |
| Subject Matter::                 |                                              |
| Suggested classification::       |                                              |
| Suggested Group Art Unit::       |                                              |
| CD-ROM or CD-R?::                | No                                           |
| Number of CD disks::             |                                              |
| Number of copies of CDs::        |                                              |
| Sequence submission?::           | No                                           |
| Computer Readable Form (CRF)?::  |                                              |
| Number of copies of CRF::        |                                              |
| Title::                          | Anti-CD38 Human Antibodies and Uses Therefor |
| Attorney Docket Number::         | 37629-0085C                                  |
| Request for Early Publication?:: | No                                           |
| Request for Non-Publication?::   | No                                           |
| Suggested Drawing Figure::       |                                              |
| Total Drawing Sheets::           | 23                                           |
| Small Entity?::                  | Yes                                          |
| Latin name::                     |                                              |
| Variety denomination name::      |                                              |
| Petition included?::             | No                                           |
| Petition Type::                  |                                              |
| Licensed US Govt. Agency::       |                                              |
| Contractor Grant Numbers::       |                                              |
| Secrecy Order in Parent Appl.?:: | No                                           |

## Applicant Information

|                                         |          |
|-----------------------------------------|----------|
| Applicant Authority Type::              | Inventor |
| Primary Citizenship Country::           | Germany  |
| Status::                                |          |
| Given Name::                            | Michael  |
| Middle Name::                           |          |
| Family Name::                           | Tesar    |
| Name Suffix::                           | Dr.      |
| City of Residence::                     | Weilheim |
| State or Province of Residence::        |          |
| Country of Residence::                  | Germany  |
| Street of mailing address::             |          |
| City of mailing address::               | Weilheim |
| State or Province of mailing address::  |          |
| Country of mailing address::            | Germany  |
| Postal or Zip Code of mailing address:: |          |

## Correspondence Information

Correspondence Customer Number:: 26633  
Name:: Heller Ehrman White & McAuliffe  
Street of mailing address:: 1666 K Street, N.W.  
Suite 300  
City of mailing address:: Washington  
State or Province of mailing address:: D.C.  
Country of mailing address:: U.S.  
Postal or Zip Code of mailing address:: 20006  
Phone number:: 202-912-2000  
  
Fax Number: 202-912-2020  
  
E-Mail address:: pbooth@hewm.com

**Representative Information**

|                                  |       |  |
|----------------------------------|-------|--|
| Representative Customer Number:: | 26633 |  |
|----------------------------------|-------|--|

- OR -

| Representative Designation:: | Registration Number:: | Representative Name:: |
|------------------------------|-----------------------|-----------------------|
|                              |                       |                       |
|                              |                       |                       |
|                              |                       |                       |
|                              |                       |                       |